PREPARATION AND EVALUATION OF RIFAMPICIN - ASCORBIC ACID LOADED PLGA NANOPARTICLES

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by
REG. NO: 26105401

Under the Guidance of

K.MOHAN KUMAR, M.Pharm.

DEPARTMENT OF PHARMACEUTICS

SWAMY VIVEKANANDHA COLLEGE OF PHARMACY

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This is to certify that the Dissertation entitled **"Preparation and Evaluation of Rifampicin - Ascorbic acid loaded PLGA nanoparticles"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, is a bonafide project work of **Reg No: 26105401,** carried out in the Department of Pharmaceutics, Swamy Vivekanandha College of Pharmacy, Tiruchengode for the partial fulfillment for the degree of Master of Pharmacy under the guidance of **K.MOHAN KUMAR, M.Pharm.,** Swamy Vivekanandha College of Pharmacy, Tiruchengode. This work is original and has not been submitted earlier for the award of any other degree or diploma of this or any other university.

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1. ABSTRACT

The aim of the present work was to minimize or prevent the degradation of rifampicin, the antitubercular drug in gastric pH condition to improve the stability and therapeutic efficacy of the drug. The study was carried out by preparing Rifampicin loaded PLGA nanoparticles using ascorbic acid as an antioxidant. Dug loaded nanoparticles were fabricated by a multistep emulsion procedure and evaluations of the prepared nanoparticles were then carried out by various methods. In this study four types of formulations were prepared. Formulation 1 (F1) is rifampicin alone loaded PLGA nanoparticles, formulation II (F2) is rifampicin – ascorbic acid (1:1) loaded PLGA nanoparticles, formulation III (F3) is rifampicin - ascorbic acid (1:2) loaded PLGA nanoparticles and formulation IV (F4) is rifampicin – ascorbic acid (1:3) loaded PLGA nanoparticles. The study concluded that ascorbic acid can minimize the degradation of rifampicin in acidic pH condition and thus improves the stability and bioavailability of rifampicin. The results also demonstrate that there is a statistically significant change in the percentage drug degradation profile when the concentration of ascorbic acid was increased.

INTRODUCTION

Tuberculosis TB is a ubiquitous, high contagious chronic granulomatous bacterial infection caused by the *Mycobacterium tuberculosis*. Tuberculosis affects one third of the world population, i.e. nearly 2 billion individuals, also responsible for 3 million death annually. India accounts for 20% of all new TB cases in the world each year .[1]

The anti-TB drugs are mainly categorized into two types namely, first line and second line drugs. First line drugs include Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PYZ), Ethambutol (ETB) and Streptomycin and second line drugs include Ciprofloxacin, Levofloxacin, Ofloxacin, Saprofloxacin, Capreomycin, Anamycin, Ethionamide, Para-amino salicylic acid, Cyclosporine and Thiacteazone.

Because of the need to take anti tubercular drugs (ATDs) daily or several times a week, there may be chance for patient noncompliance and this result in treatment failure as well as the emergence of drug resistance. Patient compliance can be improved by the use of ATD formulations which reduce the dosing frequency of the drugs. The World Health Organization (WHO) and International Union against Tuberculosis and Lung Disease (IUATLD) recommended the use of four drug fixed dose combination (FDC) to overcome these problems. The first-line drugs used in the treatment of TB are shown to have excellent potency against M. Tuberculosis. Among the four drugs, rifampicin is known to be the most unstable and recent studies have shown that this drug particularly undergoes rapid degradation in the presence of Isoniazid.

Rifampicin is a semi synthetic macrocyclic antibiotic derived from *streptomyces mediterranei* which has a unique role in killing the semi-dormant tubercle bacilli. Rifampicin acts by inhibiting mycobacterial DNA-dependent RNA polymerase synthesis by blocking RNA transcription. It is also known to have cytochrome P450 activity.[2] RIF can be hydrolyzed to even less soluble form such as 1-amino-4-methyl piperazine under acid gastric conditions. At pH-values between 7.4 and 8.2, the molecule is oxidized to an insoluble quinone derivative or a desacetylated form. The major degradation products of rifampicin are 3-formylrifamycin, rifampicin N-oxide, 25-desacetyl rifampicin and rifampicin quinine.[3]

Small molecular weight antioxidants like Vitamin C and Vitamin E plays an important role in protecting the human tissues from oxidative damage by a variety of mechanisms. Vitamin C supplements have been shown to alter many different indexes of human immune responses and the concentration of vitamin C is high in activated neutrophils and macrophages. Therefore Vitamin supplementation may prove to be beneficial.[4] .Rifampicin is well absorbed in the pH range of 1-2 even though it undergoes degradation in the acidic medium. To prevent this degradation Vitamin C can be incorporated into rifampicin-PLGA nanoparticles as an antioxidant to increase the stability of rifampicin.

For a drug molecule to reach the target site from the site of administration in sufficient concentration and to maintain therapeutic levels for a sufficient period of time, a delivery system is needed. Among the various colloidal drug delivery systems available, nanoparticles represent a very promising approach to this aim. Nanoparticles are desirable for drug delivery because of number of properties. They are known to cross the intestinal permeability barriers directly via transcellular/paracellular pathways, which explain better delivery of the encapsulated drug into the circulation.[5]. In this case, nanoparticles are expected to penetrate inside the infected cell where TB is an intracellular infection.

Among the various polymers used in drug delivery research, PLGA (poly-d,l-lactide-co-glycolide) is one of the most successfully used biodegradable nanosystem for the development of nanomedicines since it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid. Since the body can effectively deal with these two monomers, there is very minimal systemic toxicity associated with this polymer.

Thus the purpose of the present study was to prepare and evaluate Rifampicin loaded PLGA nanoparticles and an attempt was made to investigate the influence of ascorbic acid as an antioxidant on stabilizing rifampicin in the gastric environment by *In vitro* study.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Tuberculosis infection is caused by tubercle bacilli, which belong to the genus *Mycobacterium*. These form a large group, but only three relatives are obligate parasites, that can cause tuberculosis disease. They are part of the Mycobacterium tuberculosis complex and include M.tuberculosis, M.bovis and M.africanum. However generally only the first two are found in isolates from people with tuberculosis diagnosed in the U.K, with M.tuberculosis accounting for over 98% of isolates.[6]

Rifampicin is a first line anti-tubercular drug, administered orally in fixed dose combination with Isoniazid, Pyrazinamide and Ethambutol in order to overcome drug resistance to tuberculosis arising from administration of these drugs separately. However bioavailability of rifampicin is reduced owing to degradation of the drug in the stomach which is further influenced by Isoniazid which is also delivered in the stomach from fixed dose combination.

The application of microsphere technology to the treatment of the initial infection with M. tuberculosis has been studied so that multiple-drug-resistant strains do not develop. Modern drug carrier systems play an important role in controlled delivery of a pharmaceutical agent to the target at a therapeutically optimal rate and dose. Among various colloidal drug delivery systems, nanoparticles(NPs) represent a very promising approach to this aim .NPs may be defined as being submicronic colloidal systems; once in the bloodstream, surface-nonmodified NPs (conventional NPs) are rapidly opsonized and massively cleared by the fixed macrophages of mononuclear phagocyte system organs such as liver, lungs, and spleen.[7]

To understand in detail the scope for improving the stability of anti tubercular drug rifampicin, by the addition of ascorbic acid a detailed review of etiology and

epidemiology of tuberculosis, diagnosis of the disease, drug therapy in tuberculosis, new TB drugs in development, bioavailability of rifampicin from different routes of administration, degradation of rifampicin and methods attempted to prevent degradation is presented.

3.1. ETIOLOGY OF TUBERCULOSIS

Infection with tubercle bacilli occurs in the vast majority of cases by the respiratory route. The lung lesions caused by infection commonly heal, leaving no residual changes except occasional pulmonary or tracheobronchial lymph node calcification. Over 90% of people initially infected enter this latent phase, from which there is a lifelong risk of reactivation. In approximately 5-10% of apparently normal hosts and as many as 50% of people with advanced HIV infection, the initial infection may progress to pulmonary tuberculosis or , by lymphohaematogenous spread of bacilli , to pulmonary, meningeal or other extra pulmonary involvement ,or lead to disseminated disease (miliary TB).

Pulmonary (respiratory) TB is more common than extra pulmonary (non-respiratory) TB. Sites of extra pulmonary TB can include the pleura, lymph nodes, pericardium, kidneys, meninges, bones and joints, larynx, skin, intestine, peritoneum and eyes.

Symptoms include fatigue, fever, night sweats, and weight loss, which may occur early, while localizing symptoms of cough, chest pain, haemoptysis and hoarseness become prominent in the advanced stages.[6]

3.2. EPIDEMIOLOGY OF TUBERCULOSIS

Tuberculosis has afflicted the human race for centuries. The estimated annual risk of tuberculosis infection and its highest prevalence are in sub-Saharan Africa and Southeast Asia. In many industrialized countries, TB has recently failed to decline, and in Eastern Europe and the former Soviet Union, cases and deaths are increasing. Drug resistance is a serious problem, especially in the United States.[8]

In countries where the incidence of tuberculosis is stable and HIV-1 absent, a control programme reaches the WHO targets of 70% case detection and 85% cure, would reduce the incidence rate by 11% (range 8-12) per year and the death rate by 12% (9-13) per year. Without greater effort to control tuberculosis, the annual incidence of the disease is expected to increase by 41% (21-61) between 1998 and 2020 (from 7.4 million to 10.6 million cases per year). Achievement of WHO targets by 2010 would prevent 23% (15-30) or 48 million cases by 2020.[10].The average prevalence of all forms of tuberculosis in India is estimated to be 5.05 per thousand, prevalence of smear-positive cases 2.27 per thousand and average annual incidence of smear-positive cases at 84 per 1,00,000 annually.[9]

TB remains a major cause of morbidity and mortality worldwide in the 21st Century. The WHO and other organizations have put vast resources into studying the disease, as well as implementing and monitoring treatment. There are large disparities between the rates of TB in children in resource poor countries and those in industrialized countries. Factors such as poverty, overcrowding and HIV infection have contributed greatly to the resurgence of childhood TB, particularly in Sub-Saharan Africa. The mortality rates from TB in children from resource-poor counties are unacceptably high. While there are many challenges in the diagnosis and treatment of TB in children, perhaps the greatest challenge globally is to begin to identify the extent of disease in this forgotten group.[10].

Global incidence and prevalence:- WHO estimates that approximately one third of global community is infected with TB. In 2000 an estimated 2-9 million incident cases and approximately 3 million deaths are occurred worldwide due to TB. After AIDS, TB is the second most common cause of death and current trends suggest that TB will still be among 10 leading causes of global disease burden in the year of 2020.

The global distribution of TB cases is skewed heavily toward low-income and emerging economies. The highest prevalence of cases is in Asia, where China, India, Bangladesh, Indonesia, and Pakistan collectively make up over 50% of the global burden. TB cases occur predominantly (approximately 6million of the 8 million) in the economically most

productive 15-49 year old age group. About 1.7 million people died of TB in 2004, including 264000 patients who are co-infected with HIV.[11]

Effective control of tuberculosis (TB) requires an understanding of the changing epidemiology of the disease. The success in reducing the tuberculosis burden reflects several factors, including improved public health efforts, physician and patient education, infection control measures, and the use of directly observed therapy (DOT). Future efforts to curtail the incidence of TB will require vigilant public health efforts, improving education of patients and health care personnel, identifying mechanisms and routes of transmission, and assuring adequate treatment and prophylactic regimens among infected individuals.[12]

3.3. DIAGNOSIS

The preliminary diagnosis of tuberculosis disease is based on the symptoms and signs in the patient, in conjugation with skin tests, chest X-rays, sputum analysis (smear and culture) and PCR test to detect the genetic material of the causative bacteria. Microbiological investigations can confirm the diagnosis, although this can take up to 6 weeks using routinely available techniques.[6]

3.4. DRUG THERAPY IN TUBERCULOSIS

Current anti-tuberculosis chemotherapy

Since the control measures for TB such as Bacillus Chalmette- Guerin (BCG) vaccination and chemoprophylaxis appear to be unsatisfactory, treatment with anti-tubercular (anti-TB) drugs becomes the only option available. The goals of treatment are to ensure cure without relapse, to prevent death, to impede transmission, and to prevent the emergence of drug resistance. Long term treatment with a combination of drugs is required. Treatment of active TB with a single drug should never be attempted, and a single drug should never be added to a failing regimen, the result being development of MDR TB.

As suggested by WHO, treatment of TB and drug resistant cases require multi-drug therapy, comprising:

1. An initial intensive phase of rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PYZ), and Ethambutol (ETB) daily for 2 months.

2. A continuation phase of RIF and INH for a further 4 months, either daily or 3 times per week, to be administered.

INH eradicates most of the rapidly replicating bacilli in the first 2 weeks of treatment, together with streptomycin and ETB. Thereafter, RIF and PYZ have an important role in the sterilization of lesions by eradicating organisms; these two drugs are crucial for successful 6-month treatment regimens. RIF kills low or non-replicating organisms and the high sterilizing effect of PYZ serves to act on semi dormant bacilli not affected by any other anti-TB agents in sites hostile to the penetration and action of the other drugs. INH and RIF, the two most potent anti-TB drugs, kill more than 99% of tubercular bacilli within 2 months of initiation of therapy. Using these drugs in conjunction with each other reduces anti-TB therapy from 18 months to 6 months.

Tuberculosis treated with a multi-drug regimen, is thus exceptionally vulnerable to incidences of side effects, unsatisfactory patient compliance and slow improvement of patients. Therefore, despite the availability of these highly effective treatments for TB, cure rates remain low, as commercial anti-TB formulations are inconvenient to administer and patients do not take the prescribed medications with sufficient regularity and duration to achieve a cure .

Patients have to consume a large number of tablets (up to eight at one time), which is a common cause for non-compliance. It can be anticipated that non-optimal application of these short course regimens will result in the deterioration of their therapeutic potential, an escalation in the mortality rate and increased risk of developing acquired drug resistance. Resistance of *M. tuberculosis* to anti-TB agents is a worldwide problem in both Immuno competent and HIV-infected populations.[13]

3.5. NOVEL DRUG DELIVERY SYSTEMS FOR THE TREATMENT OF TUBERCULOSIS

Chemotherapy of TB is complicated by the need of multidrug regimens that need to be administered over long periods. Poor patient compliance is the single most common reason for chemotherapy failure in TB. To minimize toxicity and improve patients' compliance, extensive progressive efforts have been made to develop various implant-, micro particulate-, and various other carrier- based drug delivery systems to either target the site of *M. tuberculosis* infection or reduce the dosing frequency, which forms an important therapeutic strategy to improve patient outcomes.

Recent trends in controlled drug delivery have seen microencapsulation of pharmaceutical substances in biodegradable polymers as an emerging technology. Carriers or delivery systems such as liposomes and microspheres have been developed for the sustained delivery of anti-TB drugs and have demonstrated better chemotherapeutic efficacy when investigated in animal models (e.g. mice).Anti-TB drugs have been successfully entrapped and delivered in biodegradable polymers such as poly (DL-lactide- co-glycolide) (PLG), which are biocompatible and release drug in a controlled manner at therapeutic levels. Amidst these concerns, Ain et al. reported the pharmacokinetics of PLG encapsulated anti-TB drugs; orally administered either individually or in combination in mice. A study conducted by Pandey et al. reported the formulation of three frontline anti-TB drugs, i.e. RIF, INH and PYZ encapsulated in PLG nanoparticles. On oral administration of drug-loaded nanoparticles to *M. tuberculosis*-infected mice at every 10th day, no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment.

Therefore, oral nanoparticle-based anti-TB drug therapy can allow for a reduction in dosing frequency for better management of TB. Prabakaran et.al developed an osmotically regulated capsular multi-drug oral delivery system comprising asymmetric membrane coating- and dense semi permeable membrane coating-capsular systems for the simultaneous controlled administration of RIF and INH for the treatment of TB. This was in an attempt to reduce the problems associated with multidrug therapy. The

modified asymmetric system provided satisfactory sustained release of RIF and INH, with an initial burst release that may be sufficient to achieve minimum effective concentration in blood. Thereafter, the system provided the release of the drugs in a near zero order rate – an ideal release profile for controlled drug delivery. In turn, this would improve the safety profile of the drugs and enhance the activity duration of drugs exhibiting short half-lives. The once daily system is optimal, and could potentially enhance patient compliance.

In addition to these combinations, the past several years have seen the development of a number of RIF-only controlled release formulations for the improvement of the clinical efficacy of the drug and patient compliance. Further attempts to solve the problems inherent in multidrug therapy have included the development of biodegradable polymeric micro- or nanoparticulate carrier systems to target alveolar macrophages that harbor *M. tuberculosis.* In the case of pulmonary TB, delivering the drug directly to the site of infection through inhalation of an aerosolized delivery system has the inherent advantages of bypassing first-pass metabolism and maintaining local therapeutically effective concentrations with decreased systemic side effects.

In another approach to solve the predicament of poor patient compliance, depot-delivery of anti-TB drugs has been investigated. Studies have demonstrated that a single implant of INH in polylactic-co-glycolic acid (PLGA) copolymer could ensure sustained levels of free INH for a period of up to 8 weeks following implantation in rabbits. Gangadharam, et al.have also investigated the chemotherapy of TB in mice using single implants of INH and PYZ. Such devices, however, inherently suffer from the disadvantages of immobilization at the implantation site and surgical requirements for implantation. However, the need to develop an oral drug delivery system with improved patient acceptance is affirmed by the accelerated pace of oral drug delivery system development fostered by the need to deliver medications to patients more efficiently and with fewer side effects, especially in developing countries where controlled-delivery implants and injectable could be too expensive.[13]

Particle size, encapsulation efficiency and the structure of the composite particles are important properties with respect to pharmaceutical application. Size and structure influence the kinetics of drug release, pharmaceutical behavior and cellular uptake of the particles. Relationship between the particle properties and the conditions of preparation were studied and characterized by several authors[14]

3.6. NANOPARTICLES AS DRUG DELIVERY SYSTEMS

Over the last few decades, the applications of nanotechnology in medicine have been extensively explored in many medical areas, especially in drug delivery. Nanotechnology concerns the understanding and control of matters in the 1- 100 nm ranges, at which scale materials have unique physicochemical properties including ultra small size, large surface to mass ratio, high reactivity and unique interactions with biological systems. By loading drugs into nanoparticles through physical encapsulation, adsorption, or chemical conjugation, the pharmacokinetics and therapeutic index of the drugs can be significantly improved in contrast to the free drug counterparts.

Many advantages of nanoparticle-based drug delivery have been recognized, including improved serum solubility of the drugs, prolonging the systemic circulation lifetime, releasing drugs at a sustained and controlled manner, preferentially delivering drugs to the tissues and cells of interest, and concurrently delivering multiple therapeutic agents to the same cells for combination therapy. Moreover, drug-loaded nanoparticles can enter host cells through endocytosis and then release drug payloads to treat microbes-induced intracellular infections. As a result, a number of nanoparticle-based drug delivery systems have been approved for clinical uses to treat a variety of diseases and many other therapeutic nanoparticle formulations are currently under various stages of clinical tests.

MECHANISM OF NANOPARTICLE - BASED ANTIMICROBIAL DRUG DELIVERY TO MICROORGANISMS

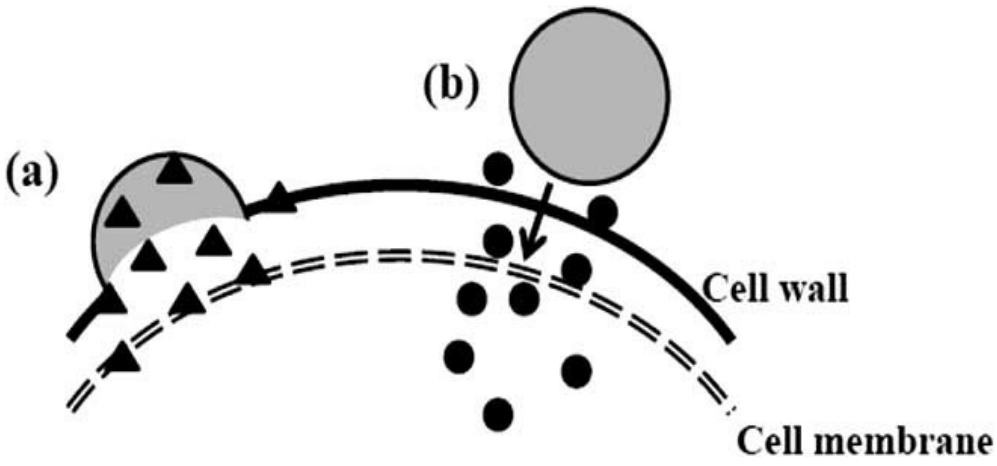


Fig. (1). (**a**) nanoparticles fuse with microbial cell wall or membrane and release the carried drugs within the cell wall or membrane; (**b**) nanoparticles bind to cell wall and serve as a drug depot to continuously release drug molecules, which will diffuse into the interior of the microorganisms.[15]

These nanocarriers are sub micron particles containing entrapped drugs intended for enteral or parenteral administration which might prevent or minimize the drug degradation and metabolism as well as cellular efflux. Nanoparticles also have a long shelf life, are made of safe materials, including synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides and have the potential for overcoming important mucosal barriers, such as intestinal, nasal and ocular barriers. [5]

3.7 POLYMERS AS DRUG DELIVERY VEHICLE

A number of polymers have been investigated for formulating biodegradable nanoparticles, such as polylactide (PLA), polycaprolactone (PCL) and poly (lactide-co-glycolide) (PLGA). These are biocompatible and biodegradable polymers which have recently been the subject of extensive investigation. However, due to copolymer crystallization, low biodegradation rate or poor flexibility, the application of polymer nanoparticles is limited. For example, in case of homopolymer poly (L-lactide), due to its

crystalline and low biodegradation rate, drug release from relevant drug delivery devices is mainly controlled by drug diffusion similar to that in non-degradable drug carriers.

Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in a polymer, leading to polymer erosion. Depending on the mode of degradation, polymeric biomaterials can be further classified into hydrolytically degradable polymers and enzymatically degradable polymers. The most part of naturally occurring polymers undergo enzymatic degradation. Biodegradation of hydrolysable polymers proceeds in a diffuse manner, with amorphous regions degrading prior to the complete split of crystalline and cross-linked regions. Factors affecting biodegradation of polymers might be: chemical structure, chemical composition, distribution of repeat units in multimers, presence of ionic groups, presence of unexpected units or chain defects, configuration structure, molecular weight, molecular weight distribution, morphology(amorphous/semi crystalline, microstructures, residual stresses),presence of low molecular-weight compounds, processing conditions, annealing, sterilization process, storage history, shape, site of implantation, adsorbed and absorbed compounds (water, lipids, ions, etc.), physicochemical factors (ion exchange, ionic strength pH), physical factors (shape and size changes, variations of diffusion coefficients, mechanical stresses, stress-and solvent-induced cracking, etc.), mechanism of hydrolysis (enzymes versus water).

Structure, properties and applications of nanoparticles are strongly affected by the properties of the polymer used in their formulation. For each application, one must evaluate the properties of the system (drug and particle) and determine the optimal formulation for a given drug delivery application. Polyesters based on polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), and their copolymers have been extensively employed as systems for drug delivery. PLGA and PLA have been approved by the FDA for numerous clinical applications, such as sutures, bone plates, abdominal mesh, and extended-release pharmaceuticals.

Biomedical uses of PLA have been reported since the 1960s. Tissue response to such biodegradable materials is characterized by minimal localized inflammation and foreign

body reaction that lessen with time. No toxic effects have been associated with the use of such polymers, biodegraded via a random, non-enzymatic process into homopolymers of lactic acid and glycolic acid, known products of cellular intermediary metabolism. PLGA degrades through hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for the degradation of PLGA is related to the ratio of monomers used in its production: the higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is copolymer with 50:50 ratios of monomers, which undergoes faster degradation (about two months) in both *in vitro* and *in vivo conditions*. Miller *et al.* have shown that PLGA 50:50 is the fastest degrading composition, with the degradation rate being decreased when either lactide or glycolide content of the copolymer was increased [16]

3.8. TB DRUGS IN DEVELOPMENT

OPC-67683

OPC-67683 is a nitromidazo-oxazole that is similar in structure to PA-824. It inhibits cell wall biosynthesis. Otsuka Pharmaceuticals (Japan) are currently conducting phase 2 clinical trials. Preclinical studies in rodents and dogs suggest that OPC-67683 could be used in HIV/AIDS as it has no effect on CYP. It may have treatment-shortening potential as it synergizes in vitro with rifampicin and pyrazinamide. OPC-67683 is effective against MDR-TB in vitro and displayed no cross-resistance to first line TB therapy. It also has potential to treat LTBI.[17]

Sudoterb LL3858

Limited data are available regarding pyrrole LL3858. It is currently undergoing phase 1 clinical trials by Lupin Limited (India). Available data suggest that LL3858 has potency against standard and drug-sensitive TB strains in vitro.[18]

SQ-109

SQ-109 is an ethylenediamine analogue of ethambutol. It is postulated to inhibit cell wall biosynthesis and has intracellular targets, which have not yet been elucidated. SQ-109 appears to be synergistic with Isoniazid and rifampicin.[19]

Gatifloxacin

Gatifloxacin is a fluroquinolone that inhibits DNA gyrase, thus inhibiting TB DNA replication and transcription. Gatifloxacin is currently undergoing phase 3 clinical trials. Gatifloxacin holds the potential to be the first TB agent to reduce pulmonary TB therapy to four-month duration. There are weak data to support its efficacy against MDR-TB.[20]

R207910

R207910 is a diarylquinoline and is also known as Compound J and TMC207. It inhibits ATP synthesis leading to ATP depletion and pH imbalance. Where it is undergoing phase 2a clinical trials in both drug sensitive and resistant disease. Murine studies suggest that R207910 has a good safety and tolerability profile and potent early bactericidal activity, matching Isoniazid. It had a synergistic effect with pyrazinamide for MDR-TB; R207910/H/Z or R207910/R/Z combinations were more effective than Amikacin/Z/Moxifloxacin/Ethionamide regimens.[21]

PA-824

PA-824 is a nitroimidazo-oxazine. It requires activation by M. tuberculosis F420 factor and inhibits synthesis of cell wall lipids as well as protein synthesis. The TB alliance is currently conducting phase 1 clinical trials of PA-824. Preliminary studies suggest that PA-824 will be active against MDR-TB and has no cross-resistance with other anti-tubercular drugs. Importantly, it is not metabolized by CYP and does not induce or inhibit

CYP. It had similar bacteriostatic efficacy to rifampicin and was more efficient than Isoniazid or moxifloxacin but less efficient than rifampicin.[22]

Moxifloxacin

Moxifloxacin is a fluroquinolone and has a similar mechanism of action to Gatifloxacin. Moxifloxacin kills rifampicin-tolerant persisters in vitro, and it may help treat MDRTB if co-administered with Ethionamide. It may thus shorten duration of TB therapy.[23]

3.9. BIOAVAILABILITY OF RIFAMPICIN

The therapeutic potential of rifampicin (RIF) in tuberculosis is well recognized due to its unique ability to kill semi dormant tubercle bacilli (*Mycobacterium tuberculosis*), when they undergo sporadic bursts of metabolism and growth [24]

After oral administration on an empty stomach, the absorption of rifampicin (rifampin) is rapid and practically complete. With a single 600mg dose ,peak serum concentration of the order of 10microgram/ml generally occurs after 2 hours of administration. The half-life of rifampicin for this dose level is of the order of 2.5 hours. Approximately 80% of rifampicin is transported in blood bound to plasma proteins, mainly albumin. Desacetylrifampicin, the more polar metabolic derivative of rifampicin, behaves in the opposite way since its rate of transfer into bile is 4 times higher than that into urine. The rate of biotransformation of rifampicin into desacetylrifampicin is of the same order of magnitude as that of biotransformation of the latter into a further metabolic derivative, which could be a glucuronide conjugate.[25]

Rifampicin is readily absorbed from the gastro- intestinal (GI) tract and showed that the pharmacokinetic parameters after intravenous infusion do not differ significantly from those after oral administration of the same doses. An absolute bioavailability of 93% after a single oral and intravenous dose of rifampicin at the beginning of the treatment of six adult patients decreases to fewer than 70% after repeated dosage due to self- induction of metabolizing enzymes by rifampicin. The Cmax after oral administration of 600 mg rifampicin averages from about 8 to 20 mg/mL. In children it can vary widely. A plasma

protein binding of 80–91% has been reported. The main metabolic pathway is deacetylation in the liver and excreted via biliary pathway. Within 24 h about 3–30% of a single oral dose is recovered in the feces. The antibiotic shows dose-dependent elimination kinetics.[26]

RIF is mainly eliminated in the bile and then reabsorbed, hence, enterohepatic circulation ensues. During this time the drug is progressively deacylated into its microbiologically active metabolite, 25-desacetyl rifampicin (DRIF) which is less absorbable as compared to the parent drug [24].The formation of different rifampicin polymorphs and the conditions for their inter-conversion has been reported. The said publication, however, gives no data to suggest that different polymorphs of rifampicin show different bioavailability. The assumption perhaps is based on a general premise that different polymorphs show different bioavailability behaviour.[27]

The absolute bioavailability of oral rifampin was determined in 20 pediatric patients. Intravenous doses of rifampin (mean 287 mg/m^2) were compared with p.o. doses (mean 324 mg/m^2). Pharmacokinetic analysis of the rifampin serum concentration data indicated that only 50 +/- 22% of a freshly prepared p.o. suspension were absorbed. The rifampicin elimination half-life following i.v. administration (2.25 +/- 0.64 h) was not different from that observed following p.o. dose administration (2.61 +/- 1.35 h). Peak rifampin concentrations were significantly higher following i.v. administration when corrected to a 300 mg/m^2 dose (27.4 vs. 9.1 [mu]g/ml, respectively, $P < 0.0001$) than after p.o administration. The peak concentration following a p.o. dose occurred at 2.0 +/- 0.9 h. The ratios of desacetylrifampicin to rifampin areas under the curves were similar for i.v. and p.o. routes of administration (0.23 vs. 0.19), suggesting linear metabolism of rifampin to this metabolite. 3-formylrifamycin SV concentrations were lower than those of desacetylrifampicin and were detectable in less than half of the patients.[28]

The bioavailability of rifampicin when administered along with antacids reduces significantly. The effect of antacids on the bioavailability of rifampicin is shown to be in the order of magnesium trisilicate > aluminium hydroxide > sodium bicarbonate, and is ascribed to the combined effects of gastric pH elevation, chelation of drug by aluminium

ions and binding of rifampicin with magnesium trisilicate.[29]Rifampicin decomposition is enhanced by the presence of Isoniazid in stomach after ingestion.80–90% of rifampicin was dissolved in 0.1 M HCl within 10 min, and all samples showed an overlapping dissolution profile.[30]

3.10. DEGRADATION OF RIFAMPICIN

Degradation of rifampicin in 0.1N HCl, and simulated gastric fluid (SGF) at 37°C in 45 min (USP dissolution test conditions) in the absence and presence of Isoniazid has been documented. Rifampicin alone decomposes in the described conditions to an average extent of 6.33%, while the loss of rifampicin in the presence of Isoniazid increases on an average to 16.32%.[31,32]

Rifampicin is well absorbed from the stomach due to its solubility, which is maximum between pH 1-2. Isoniazid is poorly absorbed from the stomach, but is well absorbed from all three segments of the intestine. In combination, rifampicin disappearance was enhanced in the presence of Isoniazid in the stomach and jejunum, but Isoniazid disappearance was not influenced by rifampicin. The study shows higher in situ rifampicin disappearance in the presence of Isoniazid, attributable to drug degradation due to catalysis by Isoniazid. As the two drugs show regional specific permeability, FDCs without reduced rifampicin bioavailability resulting from its decomposition in the presence of Isoniazid can be designed by segregating delivery of the two drugs by around 3-4 h. Rifampicin should be released in the stomach and Isoniazid in the intestine.[33]

Rifampicin is better absorbed through the stomach and duodenum than through the distal regions of the intestine. Rifampicin alone exhibited decomposition of ~4% at pH 2, whereas it was negligible at pH 5.5 and 7. Rifampicin showed similar decomposition pattern even in the presence of Nucleotide/Nucleoside Reverse Transcriptase Inhibitors (NRTIs).[2]

3.11. METHODS ADOPTED TO MINIMIZE / PREVENT DEGRADATION OF RIFAMPICIN

A study was carried out to determine the stability of rifampicin in plasma kept at an ambient temperature for 24 hrs or stored at -20^0 C for two weeks. The possible protective effect of adding ascorbic acid was studied in this. The results indicate that Rifampicin degrades rapidly in plasma at ambient temperature, and 54% loss was observed within 8hrs. This degradation can be effectively prevented by adding ascorbic acid, thus prolonging the stability for up to 12hrs. Rifampicin decomposition occurs after storage for 1 week at -20^0c. However in samples supplemented with ascorbic acid before freezing, no degradation was observed within 14 days.[34]

Rifampicin oxidizes in solution to form rifampicin quinine. Ascorbic acid is often added to solutions of RIF to slow down this oxidation and explained on short term stability studies in plasma, no degradation was observed in thawed samples up to 9 hrs. The response after 9hr were 93.7% and 96.1% of the response at t=0hr at RIF concentrations. Degradation was observed at lower concentration of the drug (0.5µg/ml) in the freeze thaw samples. However, at high concentration (20 µg/ml), this degradation was less evident.[35]

Contact between rifampicin and Isoniazid can decrease the degradation of rifampicin. Delay of rifampicin release in the acidic medium was achieved by preparing the Rifampicin-Sodium Lauryl Sulphate mixture in the ratio of 1:1 by co grinding method which is a relatively simple and effective method. Thus this approach is beneficial for the segregation of release pattern of rifampicin in alkaline environment and Isoniazid in the acidic environment of the GI tract, which will lead to prevent the degradation of rifampicin alone & its interaction with isoniazid.[36]

3.12. REVIEW OF RELEVANT WORKS

Farnaz Esmaeili et al. carried out the preparation and antibacterial activity evaluation of rifampicin-loaded poly lactide-co-glycolide nanoparticles. In this study the nanoparticles were prepared by emulsification/solvent diffusion method. The effects of several variables on the nanoparticles' characteristics were evaluated, including the amount of rifampicin, amount of poly vinyl alcohol as surfactant and internal phase volume and composition. The rifampicin encapsulation efficiency and the particle size distribution were optimized by varying these parameters. The nanoparticles were found to be spherical in shape with a relatively mono dispersed size distribution. The antibacterial activity of rifampicin against gram positive and gram negative bacteria were evaluated and found that RIF NPs could considerably improve the RIF anti bacterial efficacy.[7]

Akansha Tripathi et al. prepared and evaluated PLGA nanoparticles of the anti tubercular drug, rifampicin. In this study, the nanoparticles were prepared through single emulsion evaporation method. The rationale of this study was to develop PLGA nanoparticles loaded with rifampicin, intended to be intravenously administered and able to improve the therapeutic index of the drug. Characterization and *invitro* drug release study of the prepared nanoparticles were also carried out. The study revealed that most of the nanoparticles were fairly spherical in shape. The surface of the particles showed a characteristic smoothness. The release behavior of rifampicin exhibited a biphasic pattern characterized by an initial burst (11.26 % in 1 day) release followed by a slower and continuous release (more than 30 days). Therefore, Rifampicin loaded PLGA nanoparticles may be considered as an effective antitubercular drug delivery system for therapy.[1]

Gambhire Vaishali et al. carried out the preparation and physico-chemical evaluation of rifampicin loaded poly-(lactic-co-glycolic) acid (PLGA) nanoparticles as per 3^2 Factorial Design. PLGA (X1) and PVA (Polyvinyl alcohol) solution (X2) as a stabilizing agent were used as independent variables where Particle size (PS) (Y1), Entrapment Efficiency (EE) (Y2) and % Drug Release at 12th h (REL)(Y3) were taken as dependant variables. In this study the nanoparticles were prepared by multiple emulsion solvent

evaporation method. The results showed the method as reproducible, easy and efficient is the entrapment of drug as well as formation of spherical nanoparticles. Effect of polymer concentration was also evaluated with respect to their % drug entrapment efficiency. The *in vitro* release studies indicated that the rifampicin-loaded PLGA nanoparticles provide sustained drug release over a period of 12h. The Infrared spectroscopy analysis revealed that there was no known chemical interaction between drug and polymer. Hence, this investigation demonstrated the potential of the experimental design in understanding the effect of the formulation variables on the quality of rifampicin nanoparticles.[5]

Anjali Sharma et al. evaluated the chemotherapeutic efficacy of PLGA nanoparticles encapsulating three front line anti tubercular drugs, Rifampicin, Isoniazid, and Pyrazinamide at sub therapeutic dose against experimental tuberculosis..In this study the nanoparticles were prepared by double emulsion and solvent evaporation technique. A single oral administration of the formulation resulted in sustained drug levels in the plasma for 7-12 days and in the organs for 11-14 days with a significant improvement in mean residence time as well as drug bioavailability. The study concluded that nanoparticle based anti tubercular chemotherapy forms a sound basis for a reduction in dosing frequency and also offers the possibility of reducing the drug dosage.[37]

Kunikazu Moribe et al. prepared a drug nanoparticle formulation using ascorbic acid derivatives. Hydrophilic ascorbic acid derivatives have been used not only as food or pharmaceutical excipients but also as antioxidants. They are usually loaded into a nanoparticle formulation to prevent oxidation of the drugs and the components .In addition to drug solubilization, drug nanoparticle formation was observed using ascorbyl glycoside. Hydrophobic ascorbic acid derivatives such as ascorbylmono- and di-n-alkyl fatty acid derivatives are used either as drugs or carrier components.[38]

L.Zhang et al. carried out a study regarding the development of nanoparticles for anti microbial drug delivery. The study suggested that since the nanostructured biomaterials, particularly nanoparticles, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure, these properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional

antimicrobial therapeutics. In this, the current progress and challenges in synthesizing nanoparticle platforms for delivering various antimicrobial drugs are reviewed.[15]

Alejandro Sosnik et al. carried out a study showing the various nanotechnologies that can be applied to the treatment of tuberculosis. The study suggested that, nanotechnology is one of the most promising approaches for the development of more effective and compliant medicines. This review thoroughly overviews the state-of-the-art in the development of nano-based drug delivery systems for encapsulation and release of anti-TB drugs and discussed the challenges that are faced in the development of a more effective, compliant and also affordable TB pharmacotherapy.[39]

Zahoor Ahmad et al. evaluated the chemotherapeutic potential of Econazole and Moxifloxacin loaded PLGA nanoparticles in a study. In this, the nanoparticles were evaluated against murine TB in order to develop a more potent regimen for TB. The nanoparticles were prepared by multiple emulsion and solvent evaporation method and were administered orally to mice. A single oral dose of nanoparticles resulted in therapeutic drug concentration in plasma for upto 5 days or 4 days whilst in the organs it was upto 6 days. The study concluded that PLGA nanoparticles appear to have the potential intermittent therapy of TB, and combination of MOX, ECZ, and RIF is the most potent.[40]

Catarina Pinto Reis et al. reviewed the most important preparation methods of drug loaded polymeric nanoparticles. Advantages and disadvantages are also presented so as to facilitate selection of an appropriate nanoencapsulation method according to a particular application. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled- and sustained-release properties, sub cellular size, and biocompatibility with tissue and cells. Several methods to prepare nanoparticles have been developed during the last two decades, classified according to whether the particle formation involves a polymerization reaction or arises from a macromolecule or preformed polymer.[41]

B.N. Vedha Hari et al. thoroughly overviewed the development of novel micro particulate system, encapsulation of drug, and various other carrier-based drug delivery systems for incorporating the principal anti-TB agents. The study discussed a detailed description about the various nanotechnologies that can be applied to the treatment of TB like nanosuspension, nanoemulsion, niosomes etc.[42]

Avnesh Kumari et al. carried out a study demonstrating the various biodegradable polymeric nanoparticles based drug delivery systems. Biodegradable nanoparticles have been used frequently as drug delivery vehicles due to its grand bioavailability, better encapsulation, control release and less toxic properties. Various nanoparticulate systems, general synthesis and encapsulation process, control release and improvement of therapeutic value of nanoencapsulated drugs has been reviewed in this study. The study highlighted the impact of nanoencapsulation of various disease related drugs on biodegradable nanoparticles such as PLGA, PLA, chitosan, gelatin, polycaprolactone and poly-alkyl-cyanoacrylates.[43]

Dutt and Khuller have entrapped INH and RIF in PLG polymers. When injected subcutaneously as a single dose, the micro particles, having a diameter ranging from 11.75 μm to 71.95 μm, provided sustained release of drugs over 6–7 weeks when tested in mice. The authors previously observed that particles with a size range >10 μm remained at the site of injection forming a depot. The entrapped contents of the microparticles were gradually released by diffusion through the polymeric particles. Such depots can show release profiles extending over several months culminating in degradation of the entire polymeric device. [13]

Saranjith Singh et al. carried out a study to determine the extent of degradation of rifampicin INH and Pyrazinamide from prepared mixtures and marketed preparations containing single, two, three and four drugs under stomach conditions. The study was carried out in 0.1M HCL at 37oC for 50 mints. Under these conditions, rifampicin was decomposed by 17.8 – 24.4 %, INH to a lesser extend (3.2-4.5) and Pyrazinamide was stable. The decomposition of rifampicin was influenced by the presence of INH and not

by Pyrazinamide or Ethambutol. The results suggest that the poor bioavailability of rifampicin might be in part due to the decomposition of the drug in the stomach. [44]

Mariappan T.T et al. carried out another study to determine the reason for an increase in the decomposition of rifampicin in the presence of INH under acid condition. The degradation study was performed in 0.1M HCL at 37^{o}C in the absence and presence of INH. The study concluded that the degradation of rifampicin was increased approximately three fold in the presence of INH.[45]

Le Guellec et al. developed an HPLC assay method to monitor rifampicin plasma concentration that was used to study the possible degradation of rifampicin in plasma sample. The report described the stability of rifampicin in plasma kept at an ambient temperature for 24hrs or stored at - 20^{o}c for 2 weeks. The possible protective effect of adding ascorbic acid was also studied. The results indicated that rifampicin degraded rapidly in plasma at an ambient temperature and a 54 % loss was observed within 8 hours. However in samples supplemented with ascorbic acid before freezing, no degradation was observed within 14 days and thus the authors suggested that decomposition of rifampicin can be effectively prevented by adding ascorbic acid thus prolonging the stability.[46]

3.13. BACKGROUND OF THE STUDY

The literature survey thus reveals the importance of fixed dose combination of rifampicin, Isoniazid, Pyrazinamide and Ethambutol in the treatment of TB to overcome the drug resistance and to improve the patient compliances. Rifampicin appears to be the best choice for the treatment of TB however it has poor bioavailability from FDC formulation following oral administration due to degradation in the stomach. Rifampicin is well absorbed in the pH range of 1-2 even though it undergoes degradation in the acidic medium and the degradation of rifampicin increases in the presence of Isoniazid and thus affects bioavailability of rifampicin. Development of any method that can stabilize rifampicin against degradation in the stomach will be therapeutically beneficial.

Since nanoparticles are known to cross the intestinal permeability barriers directly via transcellular / paracellular pathways, it offers better delivery of the encapsulated drug into the circulation. In this case they are expected to penetrate inside the infected cell, where TB is an intracellular infection. From the literature study it was found that PLGA is one of the most successfully used biodegradable nanosystem for the development of nanomedicine since it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid. Based on these considerations the present study attempted to improve the stability of rifampicin - PLGA nanoparticles using ascorbic acid as an antioxidant.

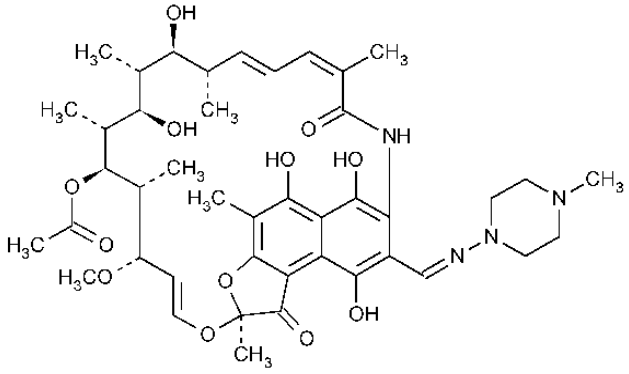
DRUG PROFILE

4. DRUG PROFILE

4.1. Name of drug: - Rifampicin

Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semi synthetic compound derived from *Amycolatopsis rifamycinica* (formerly known as *Amycolatopsis mediterranei* and *Streptomyces mediterranei*).

Structure:



Rifampicin

Empirical formula: $C_{43}H_{58}N_4O_{12}$

Molecular weight: 822.94.

Chemical name: 3-[[(4-methyl-1-piperazinyl)imino]methyl]- .
5,6,9,17,19,21- Hexahydroxy-23-methoxy-
2,4,12,16,18,20,22-heptamethyl-8-[N-(4-methyl-1-piperazinyl)formimidoyl]-2,7-

(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-b]furan
-1,11-(2H)-dione 21-acetate [13292-46-1].

Physical and Chemical Properties

Appearance:	orange-red powder
Melting point:	183-188°C
Solubility:	Soluble in dilute acid solutions, slightly soluble in water

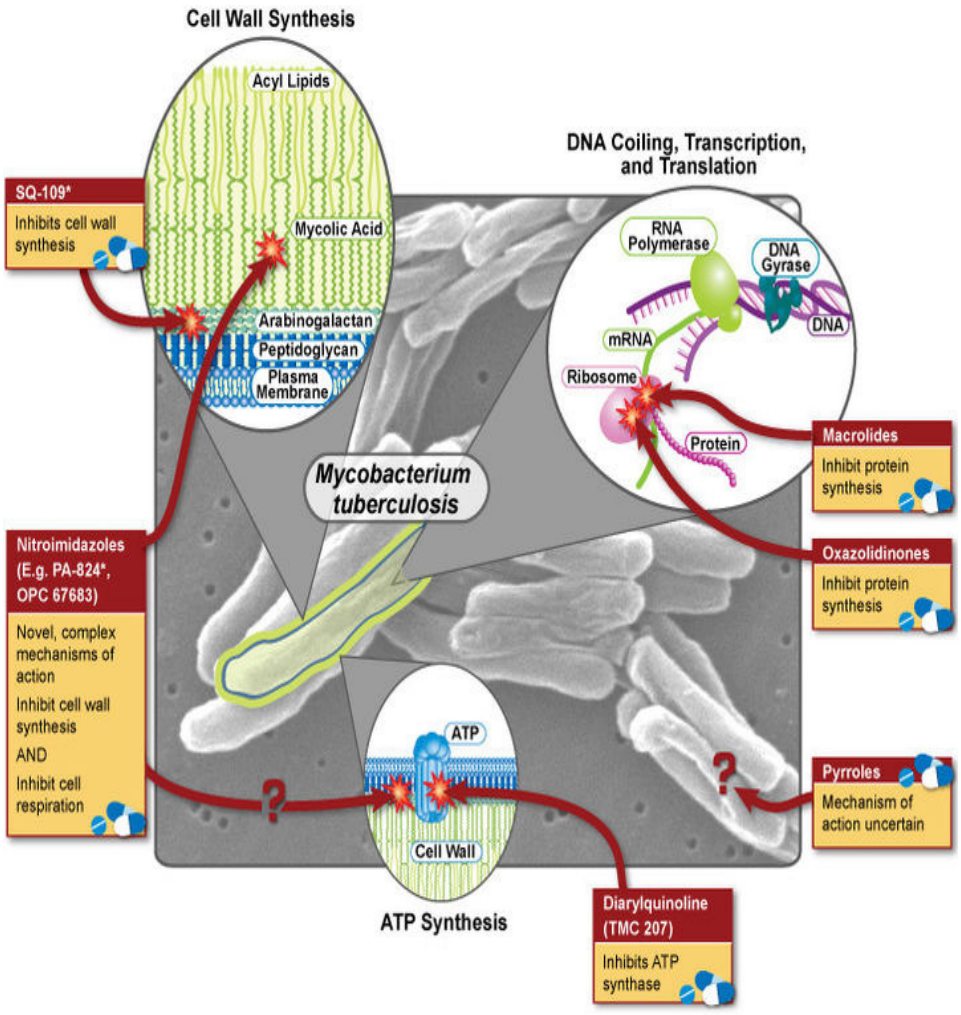
Interactions:

1. Antacids containing aluminium hydroxide reduces the bioavailability of rifampicin
2. Food lowers peak blood levels because of interference with absorption of rifampicin.
3. Para-amino salicylic acid granules may delay rifampicin absorption. These two drugs should be given 8 to 12 hours apart.
4. Isoniazid and rifampicin interaction has led to hepatotoxicity.
5. Alcohol intake with rifampicin increases the risk for hepatotoxicity.
6. Rifampicin induces microsomal enzymes of the liver and therefore accelerates metabolism of some drugs, e.g. beta blockers, oral contraceptive pills etc.
7. When rifampicin and oral contraceptives are used concomitantly, there is decreased effectiveness of oral contraceptives. It was reported that rifampicin may be the cause of some menstrual disorders when used with oral contraceptive pills.
8. Rifampicin can lower the plasma calciferol (Vitamin D) level because of induction of enzyme activity.
9. Barbiturates and salicylates decrease the activity of rifampicin.

Rifampicin : Mechanism of Action and Resistance[47]

Rifampin specifically inhibits bacterial RNA polymerase, the enzyme responsible for DNA transcription, by forming a stable drug-enzyme complex with a binding constant of 10-9 *M* at 37°C. Various steps involved in transcription of bacteria are inhibited when rifampin interferes with RNA polymerase. The various steps in the process of transcription of DNA to RNA are (1) binding of the enzyme to DNA; (2) binding of the first nucleoside tri-phosphate to the enzyme-DNA complex; (3) formation of the first phosphodiester bond, leading to a dinucleotide (chain initiation); (4) assembly of more nucleotides to form a polyribonucleotide (chain elongation); and (5) release of the completed RNA chain from the template (chain termination). More specifically, the β subunit of this complex enzyme is the site of action of drug. The corresponding mammalian enzymes are not affected by rifampin.

Bacterial resistance to rifampin is caused by mutations leading to a change in the structure of the β subunit of RNA polymerase. Such resistance is not an all-or nothing phenomenon; rather, a large number of RNA polymerases with various degrees of sensitivity to rifampin have been found. No strict correlation exists between enzyme sensitivity and MIC values, since inhibition of RNA synthesis does not always show up to the same extent in the two different test systems used for the determination of these values.



Mechanism of action of Rifampicin on Mycobacterium tuberculosis (Figure 2)

Formulations of rifampicin in market

Monocomponent products

Rifampicin capsules of 150 mg, 300 mg, 450 mg, 600 mg

Combination Products

Dipicin Isoniazid 150 mg, Rifampicin 300 mg

Pyrina	Isoniazid 150 mg, Rifampicin 150 mg, Pyrazinamide 500 mg
Rambutol	Isoniazid 200 mg, Ethambutol HCl 400 mg, Rifampicin 300 mg, Pyridoxine 25 mg
Rimactazid	Isoniazid 100 mg Rifampicin 150 Isoniazid 200 mg Rifampicin 225 Isoniazid 150 mg Rifampicin 300

Routes of entry

Oral: This is the common route of entry.

Inhalation: Not applicable.

Dermal: Not applicable

Eye: Use for ocular chlamydial infection treatment

Parenteral: Rifampicin may be given intravenously.

Pharmacokinetics:-

Absorption:

Rifampicin is readily absorbed from the gastrointestinal tract (90%). Peak plasma concentration occurs at 1.5 to 4 hours after an oral dose. After a 450 mg oral dose, plasma levels reach 6 to 9 µg/mL while a 600 mg dose on an empty stomach yields 4 to 32 µg/mL (mean 7 µg/mL).

Distribution:

It is widely distributed in body tissues and fluids. 89% of rifampicin in circulation is bound to plasma proteins. It is lipid soluble. When the meninges are inflamed, rifampicin enters the cerebrospinal fluid (4.0 µg/mL after a 600 mg oral dose). It reaches at therapeutic levels in the lungs, bronchial secretions, pleural fluid, other cavity fluid, liver, bile, and urine. Rifampicin has a high degree of placental transfer with a fetal to maternal serum level ratio of 0.3. It is distributed into breastmilk.[45]. The apparent volume of distribution (VD) is 0.93 to 1.6 L/kg.

Biological half-life by route of exposure:

T 1/2 = three hours range (2 to 5 hours).

Metabolism:

Approximately 85% of rifampicin is metabolized by the liver microsomal enzymes to its main and active metabolite-deacetylrifampicin. Rifampicin undergoes enterohepatic recirculation but not the deacetylated form. Rifampicin increases its own rate of metabolism. Rifampicin may also be inactivated in other parts of the body. Formyl rifampicin is a urinary metabolite that spontaneously forms in the urine.

Elimination:

Rifampicin metabolite deacetylrifampicin is excreted in the bile and also in the urine. Approximately 50% of the rifampicin dose is eliminated within 24 hours and 6 to 30% of the drug is excreted unchanged in the urine, while 15% is excreted as active metabolite. Approximately 43 to 60% of oral dose is excreted in the faeces.

Intrinsic total body clearance is 3.5 (+/- 1.6) mL/min/kg, reduced in kidney failure. Renal clearance is 8.7 mL/min/kg. Rifampicin is excreted in breast milk (1 to 3 µg/ml).

Uses

- The primary indications for rifampicin are for treatment of tuberculosis (pulmonary and extra pulmonary lesions) and for leprosy.
- It has some anti chlamydial activity and in vitro activity against some viruses (pox viruses and adenoviruses.
- It has recently been used for brucellosis.

Adverse Effects

- Severe gastrointestinal side-effects (e.g.pseudomembranouscolitis).
- Hypersensitivity, shock, shortness of breath, acute hemolytic anemia, renal failure (nephrotoxicity).
- The other adverse effects are staining of body fluids, rash, chills and fever, nausea and vomiting, arthralgia, diarrhea, and peripheral neuritis.
- Ocular side effects as a consequent to rifampicin use occur in 5 to 15% of patients.
- Angioneurotic edema, urticaria, purpura, Stevens-Johnson syndrome, exfoliative dermatitis or pemphigoid lesions have been reported.
- Local ophthalmic use of rifampicin has caused irritation of the eyes which manifests transiently as lacrimation, hyperemia, edema and ocular pain in Special risks

Toxicity

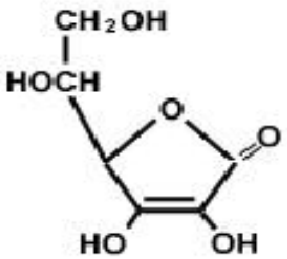
- Thrombocytopenia
- Hemolysis
- Renal failure.
- Flu-like syndrome

4.2 ASCORBIC ACID

Category : Water soluble vitamin

Empirical formula : $C_6H_8O_6$

Structure :



Chemical name : L-ascorbic acid

Molecular weight : 176.1

Melting point : about 190°C (with decomposition)

Colour : white to slightly yellowish crystalline powder

Taste : slight acidic taste.

Solubility : freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, in ether, and in benzene.

Dosage and Administration:-

Ascorbic acid (vitamin c) is usually administered orally. When oral administration is not feasible or when malabsorption is suspected, the drug may be administered IM, IV, or subcutaneously. When given parenterally, utilization of the vitamin reportedly is best after IM administration and that is the preferred parenteral route.The average protective dose of vitamin C for adults is 70 to 150 mg daily. In the presence of scurvy, doses of 300 mg to 1 g daily are recommended. However, as much as 6 g has been administered parenterally to normal adults without evidence of toxicity.

Daily intake of dietary vitamin C (according to U.S. recommended dietary allowances), are listed below.

Pediatric

- Birth - 6 months: 40 mg
- Infants 6 - 12 months: 50 mg

- Children 1 - 3 years: 15 mg
- Children 4 - 8 years: 25 mg
- Children 9 - 13 years: 45 mg
- Adolescent girls 14 - 18 years: 65 mg
- Adolescent boys 14 - 18 years: 75 mg

Adult

- Men over 18 years: 90 mg
- Women over 18 years: 75 mg
- Breastfeeding women: 120 mg

Because smoking depletes vitamin C, people who smoke generally need an additional 35 mg per day.

The dose recommended to prevent or treat many of the conditions mentioned in the Uses section is often 500 - 1,000 mg per day.

Pharmacokinetics:-

Absorption, transport, and disposal

Ascorbic acid is absorbed in the body by both active transport and simple diffusion. Sodium-Dependent Active Transport—Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs)—are the two transporters required for absorption. SVCTs appear to be the predominant system for vitamin C transport in the body.

With regular intake the absorption rate varies between 70 to 95%. However, the degree of absorption decreases as intake increases. At high intake (12g), fractional human absorption of ascorbic acid may be as low as 16%; at low intake (<20 mg) the absorption rate can reach up to 98%.Although the body's maximal store of vitamin C is largely determined by the renal threshold for blood, there are many tissues that maintain vitamin C concentrations far higher than in blood. Biological tissues that accumulate over 100

times the level in blood plasma of vitamin C are the adrenal glands, pituitary, thymus, corpus luteum, and retina. Those with 10 to 50 times the concentration present in blood plasma include the brain, spleen, lung, testicle, lymph nodes, liver, thyroid, small intestinal mucosa, leukocytes, pancreas, kidney and salivary glands.

Ascorbic acid can be oxidized (broken down) in the human body by the enzyme L-ascorbate oxidase. Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by this enzyme and removed.

Mechanism of Antioxidants

Ascorbic acid is a mild reducing agent. For this reason, it degrades upon exposure to oxygen, especially in the presence of metal ions and light. It can be oxidized by one electron to a radical state or doubly oxidized to the stable form called dehydroascorbic acid.

Ascorbate usually acts as an antioxidant. Typically it reacts with oxidants such reactive oxygen species, such as the hydroxyl radical formed from hydrogen peroxide. Such radicals are damaging to animals and plants at the molecular level due to their possible interaction with nucleic acids, proteins, and lipids. Sometimes these radicals initiate chain reactions. Ascorbate can terminate these chain radical reactions by electron transfer. Ascorbic acid is special because it can transfer a single electron, owing to the stability of its own radical ion called "semidehydroascorbate". dehydroascorbate.

The net reaction is:

$$RO\bullet + C_6H_7O_6^- \rightarrow ROH + C_6H_6O_6\text{-}\bullet.$$

The oxidized forms of ascorbate are relatively unreactive, and do not cause cellular damage.

However, being a good electron donor, excess ascorbate in the presence of free metal ions can not only promote, but also initiate free radical reactions, thus making it a potentially dangerous pro-oxidative compound in certain metabolic contexts.

Functions

- Converts Folic Acid into active form Folinic Acid
- Essential for the formation of intercellular material, bone and teeth
- Essential for the absorption of iron
- Fights bacterial and viral infections Antioxidant which helps defend cells from the effects of smoke, pollution and other highly reactive substances called free radicals
- Controls blood cholesterol levels
- Maintains healthy reproductive organs
- May help protect against certain cancers, cataracts and heart disease
- Necessary in production of red blood cells
- Prevents allergic reactions (antihistamine activity)
- Prevents hemorrhaging

Precautions:

Vitamin C supplements have a diuretic effect, so should drink plenty of fluids when taking them.

Most commercial vitamin C is made from corn. People sensitive to corn should look for alternative sources, such as sago palm.

Vitamin C increases the amount of iron absorbed from foods. People with hemochromatosis (an inherited condition where too much iron builds up in the body) should not take vitamin C supplements.

While vitamin C is generally considered safe because your body gets rid of what it does not use, in high doses (more than 2,000 mg daily) it can cause diarrhea, gas, or stomach upset. If you experience these side effects, lower the dose of vitamin C.

People with kidney problems should talk to their doctor before taking vitamin C.

People who smoke or use nicotine patches may need more vitamin C because nicotine decreases the effectiveness of vitamin C in the body.

Infants born to mothers taking 6,000 mg or more of vitamin C may develop rebound scurvy because their intake of vitamin C drops after birth. If you are pregnant, talk to your doctor before taking more than 1,000 mg of vitamin C.

Possible Interactions:

- **Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs)** -- Both aspirin and NSAIDs can lower the amount of vitamin C in the body because they cause more of the vitamin to be lost in urine. In addition, high doses of vitamin C can cause more of these drugs to stay in the body, raising the levels in your blood. Some very early research suggests that vitamin C might help protect against stomach upset that aspirin and NSAIDs can cause.

- **Acetaminophen (Tylenol)** -- High doses of vitamin C may lower the amount of acetaminophen passed in urine, which could cause the levels of this drug in your blood to rise.

- **Barbiturates** -- Barbiturates may decrease the effects of vitamin C. These drugs include Phenobarbital (Luminal), pentobarbital (Nembutal), and seconobarbital (Seconal).

- **Chemotherapy drugs** -- As an antioxidant, vitamin C may interfere with the effects of some drugs taken for chemotherapy

- **Nitrate medications for heart disease** -- The combination of vitamin C with nitroglycerin, isosorbide dinitrate (Isordil), or isosorbide mononitrate (Ismo) reduces the body's tendency to build up a tolerance to these medications so that they no longer work

- **Oral contraceptives (birth control pills) and hormone replacement therapy (HRT)** – Oral estrogens can decrease the effects of vitamin C in the body.

- **Protease inhibitors** -- Vitamin C appears to slightly lower levels of indinavir (Crixivan), a medication used to treat HIV and AIDS.

- **Tetracycline --** Taking vitamin C with the antibiotic tetracycline may increase the levels of this medication; it may also decrease the effects of vitamin C in the body. Other antibiotics in the same family include minocycline (Minocin) and doxycycline (Vibramycin). [48]

Toxicity

- Unpleasant diarrhea
- Gastrointestinal disturbances
- Hyperoxaluria
- Hemolysis

Uses

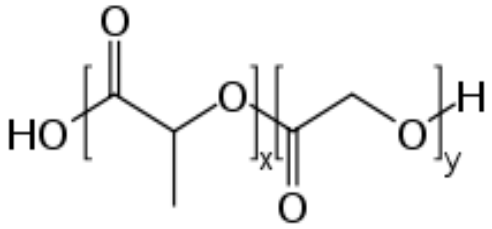
- Treatment of Scurvy
- Stabilizes food and plasma
- Good antioxidant
- Treating lead, mercury and cadmium poisoning.
- Reduces gastric cancer
- Bleeding gums

POLYMER PROFILE

5. POLYMER PROFILE

Name of the polymer : - PLGA or poly (lactic-*co*-glycolic acid)

Structure



Structure of poly(lactic-*co*-glycolic acid). *x*= number of units of lactic acid; *y*= number of units of glycolic acid.

Typical properties

PLGA or **poly (lactic-*co*-glycolic acid)** is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. Depending on the ratio of lactide to glycolide used for the polymerization, different forms of PLGA can be obtained. All PLGAs are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60 °C. Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.

The mechanical strength, swelling behavior, capacity to undergo hydrolysis and, subsequently, the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The resultant crystallinity of the PLGA co-polymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in

the copolymer chain. PLGA polymers containing a 50: 50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing a higher proportionof either of the two monomers.

PGA is highly crystalline because it lacks the methyl side groups of the PLA. Lactic acid is more hydrophobic than glycolic acid and, therefore, lactide-rich PLGA co-polymers are less hydrophilic, absorb less water and, subsequently, degrade more slowly.

It has a glass transition temperature (Tg) of 45°C and an inherent viscosity of 0.5-0.8 mPa. The Tgs of the PLGA co-polymers are above the physiological temperature of 37°C and hence they are normally glassy in nature. Thus, they have a fairly rigid chain structure, which gives them significant mechanical strength to be formulated as a degradable device. It has been reported that the Tgs of PLGA decrease with the decrease of lactide content in the co-polymer composition with decreasing M.W.

Biodegradation of PLGA

In both *in vitro* and *in vivo,* the PLGA co-polymer undergoes degradation in an aqueous environment (hydrolytic degradation or biodegradation) through cleavage of its backbone ester linkages. The polymer chains undergo bulk degradation and the degradation generally occurs at a uniform rate throughout the PLGA matrix. It has been recorded that the PLGA biodegradation occurs through random hydrolytic chain scissions of the swollen polymer. The carboxylic end groups present in the PLGA chains increase in number during the biodegradation process as the individual polymer chains are cleaved. These are known to catalyze the biodegradation process. It has also been reported that large fragments are degraded faster internally and amorphous regions degrade faster than crystalline regions. The biodegradation rates of the PLGA co-polymers are dependent on the molar ratio of the lactic and glycolic acids in the polymer chain, M.W. of the polymer and the degree of crystallinity

Applications

The possibility to tailor the polymer degradation time by altering the ratio of the monomers used during synthesis has made PLGA a common choice in the production of a variety of biomedical devices such as: grafts, sutures, implants, prosthetic devices, micro and nanoparticles. They are also easy to formulate into various delivery systems for carrying a variety of drug classes, such as vaccines, peptides, proteins and micro molecules, which have been approved by the Food and Drug Administration for drug delivery use.

It has been used successfully in delivery of Amoxicillin in treating listeriosis (treatment of Listeria monocytogenes infection).As an example, a commercially available drug delivery device using PLGA is Lupron Depot® for the treatment of advanced prostate cancer.[49]

AIM AND OBJECTIVE

6. AIM AND OBJECTIVE

Rifampicin when administered alone or in combination with Isoniazid, Pyrazinamide and Ethambutol degrades in the stomach and results in poor bioavailability which makes difficulties in effectively controlling tuberculosis. Development of any method that can stabilize rifampicin against degradation in the gastric environment will be therapeutically beneficial.

Aim and objective of the present study is:-

1) To prepare rifampicin – PLGA nanoparticles using ascorbic acid as an antioxidant to improve the stability of rifampicin.

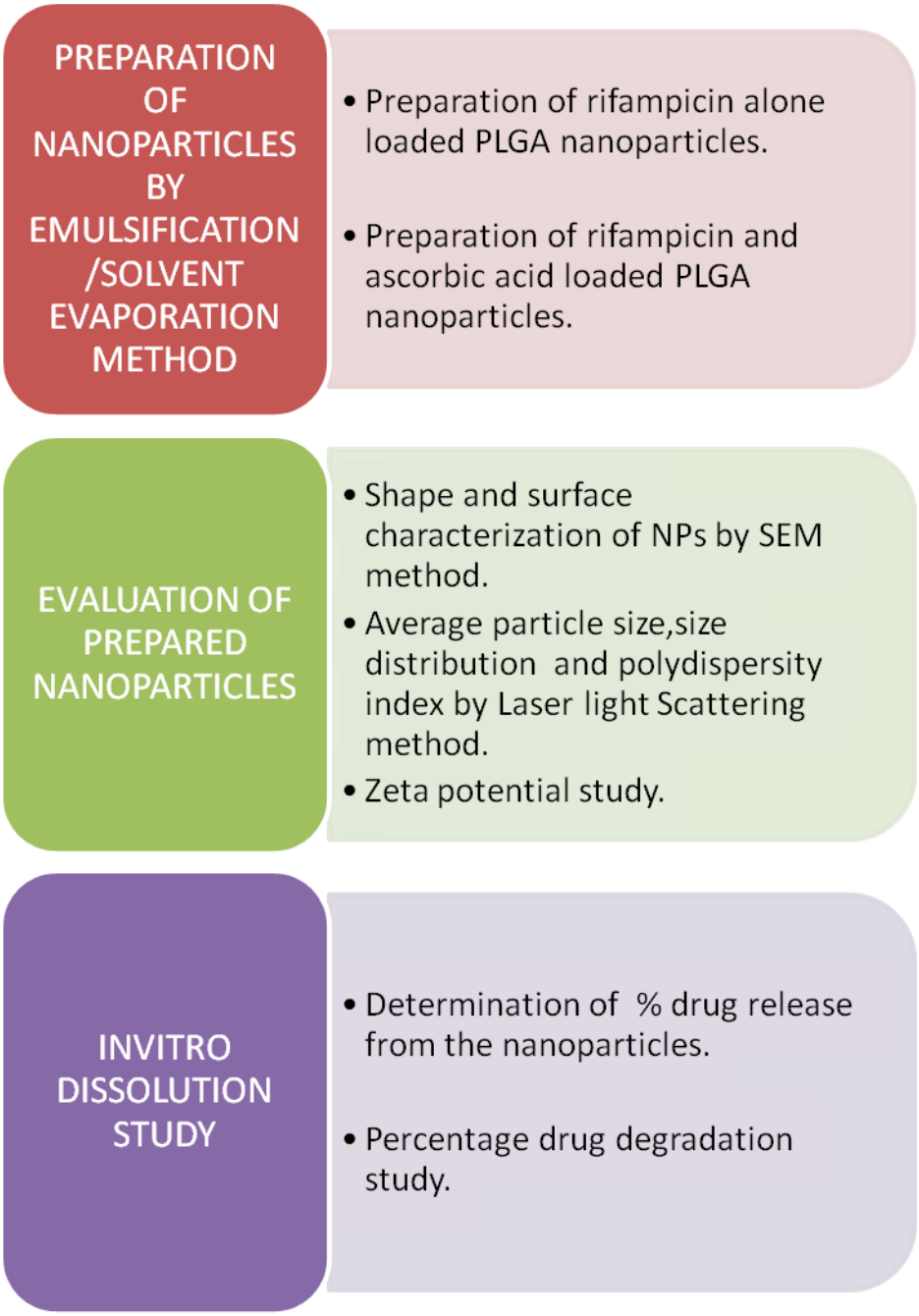
2) Evaluation of the prepared nanoparticles.

- Shape and Surface morphology of the prepared nanoparticles (SEM)
- Particle size and size distribution
- Polydispersity Index
- Zeta potential study
- Statistical analysis

3) To carry out the *in-vitro* dissolution study of the prepared nanoparticles to observe the degradation of rifampicin alone and with ascorbic acid of different concentrations in pH 1.2 medium to investigate whether ascorbic acid can improve the stability of rifampicin in gastric pH condition and so improve the bioavailability of rifampicin.

PLAN OF WORK

7.PLAN OF WORK



MATERIALS AND EQUIPMENTS

8. MATERIALS AND EQUIPMENTS

8.1. Materials used

Sl.No:	Materials	Manufactured/ Supplied by
1.	Rifampicin	Astha Laboratories,India
2.	Ascorbic acid	Qualigens Fine chemicals.Mumbai
3.	P LGA	Boehringer Ingelheim Pharma
4.	Dichloromethane	Nice chemicals Pvt.Ltd.Mumbai
5.	Polyvinyl alcohol	Molychem.India

8.2. Equipments used

Sl.No:	Equipments	Manufactured/Supplied by
1.	Magnetic stirrer	Elektrocrafts,Mumbai
2.	Ultrasonicator	Bandelin Sonoplus model HD
3 .	UV/Visible spectrophotometer	Perkin Elmer Lambda-25
4.	Single pan Digital Balance	Shimadzu ELB 300
5.	Dissolution test apparatus-USP	Veego apparatus,Chennai
6.	Scanning Electron Microscope	Philips XL 30,Eindhoven
7.	Laser Particle size analyzer	Malvern Instruments UK
8.	Zetasizer	Malvern Instruments UK

METHODOLGY

9. METHODOLOGY

9.1. PREPARATION OF NANOPARTICLES

Rifampicin and ascorbic acid loaded PLGA nanoparticles were fabricated by an Emulsification/solvent evaporation method, which involved the formation of stable emulsion and evaporation of organic solvent by continuous stirring. The study was carried out by preparing four types of formulations.

Formulation 1 (F1) is rifampicin alone loaded PLGA nanoparticles, formulation II (F2)is rifampicin –ascorbic acid(1:1) loaded PLGA nanoparticles, formulation III (F3)is rifampicin -ascorbic acid (1:2) nanoparticles and formulation IV (F4)is rifampicin – ascorbic acid (1:3) loaded nanoparticles. In all the cases, drug: polymer ratio was taken as 1:1 and ascorbic acid was taken in three different ratios as shown in Table 1

Procedure:-

Drug loaded PLGA nanoparticles were prepared by a multistep emulsion procedure.50 mg of rifampicin and required quantities of ascorbic acid were accurately weighed and added to 10ml of dichloromethane containing the polymer [drug : polymer ratio was taken as (1:1)]. Distilled water was emulsified in the DCM containing drug and polymer to form w/o primary emulsion. It was then emulsified by sonication for 15 minutes. Primary emulsion was then poured into 8ml of 1%w/v aqueous Poly Vinyl Alcohol solution and stirred using a magnetic stirrer to form the second w/o/w multiple emulsion. The latter was then stirred continuously overnight for the complete removal DCM. The nanoparticles were then recovered by centrifugation (9000 -10,000 rpm for 15 minutes), washed thrice with distilled water and vaccum dried.[37]

Table 1

FORMULATION CODE	INGREDIENTS
F0	PURE RIFAMPICIN
F1	RIFAMPICIN+PLGA(1:1)
F2	RIF+PLGA+ASC(1:1:1)
F3	RIF+PLGA+ASC(1:1:2)
F4	RIF+PLGA+ASC(1:1:3)

9.2. EVALUATION OF THE PREPARED NANOPARTICLES

Characterization of the prepared nanoparticles was then carried out. It includes determination of particle size, size distribution, shape, surface morphology, Poly dispersity Index and zeta potential. Scanning electron microscopy was used to determine the shape and surface morphology of the nanoparticles. Average particle size and polydispersity index of nanoparticles were measured by Laser light scattering method. Zeta potential of the nanoparticles was determined using a zetasizer.

Shape and surface morphology of nanoparticles [1]

The morphology of Rifampicin –ascorbic acid loaded PLGA nanoparticles were analyzed using a scanning electron microscope. Samples were prepared from dilutions in distilled water of particle suspensions and dropped onto stubs using double sided sticking tape.

After air drying, particle were coated with a thin layer of platinum film and then examined by scanning electron microscopy.

Particle size characterization of the nanoparticles [7]

The particle size, size distribution and poly dispersity index of the nanoparticles were measured by a laser particle size analyzer after suitable dilutions.

Zeta Potential Study [1]

The surface charge of nanoparticles was determined by the electrophoretic mobility of nanoparticles in a U type tube at 25˚C, using a zetasizer .

9.3. *INVITRO* RELEASE STUDY [5]

A solution of 0.1N HCL was placed in the vessel of USP dissolution apparatus type 2 (US Pharmacopoeia XXIII, 1995) with rotating paddle at 100rpm and the temperature was maintained at 37±0.2ºc.RIF loaded PLGA nanoparticles with ascorbic acid of different ratios were accurately weighed, dissolved in and diluted to 100ml with 0.1N HCL. The resulting solution was transferred immediately to the dissolution bath. Specimens were withdrawn at 15 min, 30min and 60min.An aliquot, 0.5ml, 1ml, 2 ml, 3ml, 4 ml and 5ml were extracted immediately with 100ml of pH 1.2 medium using a cyclomixer. Samples were analyzed spectrophotometrically at 475nm[1,7] and the percentage degradation was calculated using the given formula[2]

$$\% \text{ Degradation loss} = \frac{\text{Initial concentration - Final concentration}}{\text{Initial Concentration}} \times 100$$

9.4. PROCEDURE FOR PREPARATION OF pH 1.2 BUFFER:

Preparation of pH 1.2: 50ml 0f 0.2M KCl is mixed with 85ml of 0.2M HCl and make up to 200ml with water.

Note:

0.2M KCl: 14.911gm of KCl was dissolved in H_2O and dilute with water and made up to 1000ml.

0.2M HCl: 17ml of HCl was mixed with 1000ml 0f H_2O. [50]

Preparation of standard stock solution:

100mg Rifampicin PLGA nanoparticles were dissolved in 100ml pH 1.2 solution. From this required quantities were taken for further dilution process.

Dilution process:

↓

0.5ml→100ml (5 µg)
1ml → 100ml (10 µg)
2ml → 100ml (20 µg)
3ml → 100ml (30 µg)
4ml → 100ml (40 µg)
5ml → 100ml (50 µg)

Standard calibration curve for Rifampicin + Ascorbic acid (1:1) nanoparticles at pH 1.2:

100 mg of rifampicin - ascorbic acid nanoparticles (1:1) were accurately weighed and dissolved in 100ml of pH 1.2 buffer.

RESULTS

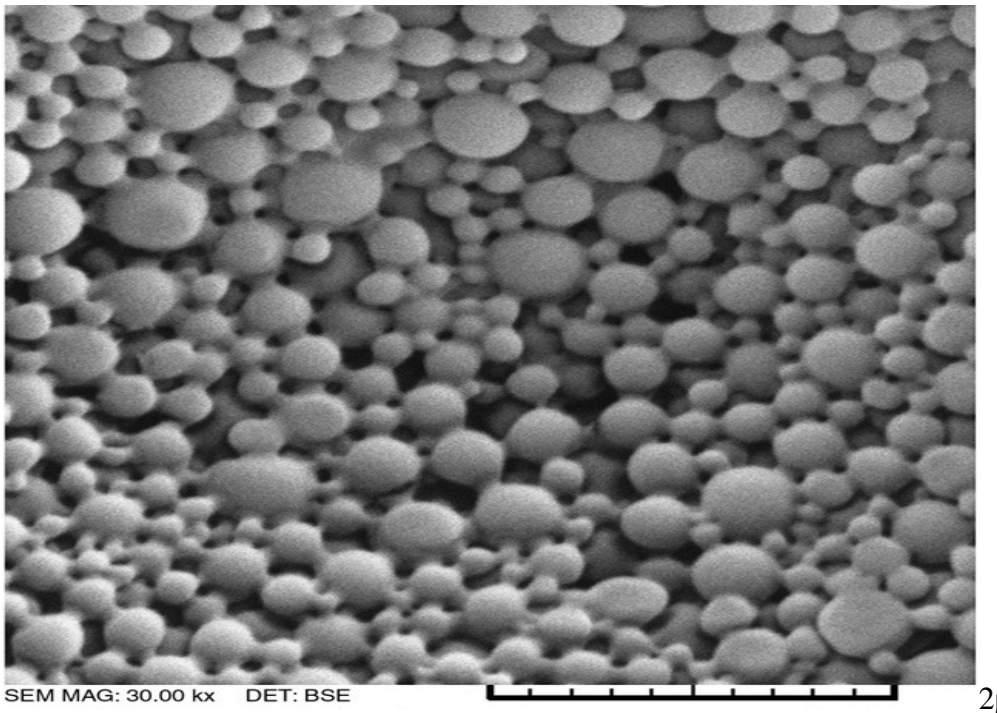
10. RESULTS

10.1. SHAPE AND SURFACE CHARACTERIZATION OF THE PREPARED NANOPARTICLES [SEM]

Scanning electron micrograph of the prepared Rifampicin - Ascorbic acid loaded PLGA nanoparticles are shown in Figure 3

SEM images revealed that the nanoparticles were spherical with smooth surface and they are relatively mono dispersed.

Figure - 3


SEM MAG: 30.00 kx DET: BSE
2µm
HV: 20.0 kV
VAC: HiVac
Device: MV2300
Obducat CamScan

10.2. PARTICLE SIZE CHARACTERIZATION OF THE NANOPARTICLES

The mean particle size and polydispersity Index of all the samples were determined (Table 2)

Table 2

S.NO	FORMULATIONS	MEAN DIAMETER(nm)±SD	PdI
1.	RIFAMPICIN+PLGA(1:1)	375± 20	0.312
2.	RIF+PLGA+ASC(1:1)	378 ± 22	0.316
3.	RIF+PLGA+ASC(1:2)	374 ± 18	0.308
4.	RIF+PLGA+ASC(1:3)	380 ± 23	0.317

Laser particle size analyzer yields the diameter of the bulk population. Particles were in the size range of 374-380 ± 18-23 (SD) nm. Polydispersity index is a measure of the distribution of particles in a given polymer sample. It gives the distribution range from 0.000 to 0.500.Polydispersity index greater than 0.5 indicates aggregation of particles. Here it is in the range of 0.308 - 0.317.

10.3. ZETA POTENTIAL STUDY

Zeta potential is a term related to the stability of samples. For molecules and particles that are small enough, high zeta potential will confer stability i.e. it resist aggregation. Here zeta potential of the prepared nanoparticle was found to be -46.6, which would not allow aggregation.

10.4. *IN-VITRO* STABILITY STUDY

Standard curves of rifampicin alone and in combination with ascorbic acid in different ratios at pH 1.2 buffer (Table 3)

Table -3

Conc. in µgm	Rif	Rif + PLGA NPs	Rif+asc(1:1) NPs	Rif+asc(1:2) NPs	Rif+asc(1:3) NPs
0	0	0	0	0	0
5	0.082	0.084	0.090	0.052	0.054
10	0.160	0.162	0.140	0.103	0.093
20	0.305	0.320	0.280	0.192	0.181
30	0.456	0.473	0.422	0.280	0.273
40	0.607	0.633	0.561	0.381	0.352
50	0.759	0.801	0.701	0.475	0.446

Figure 4

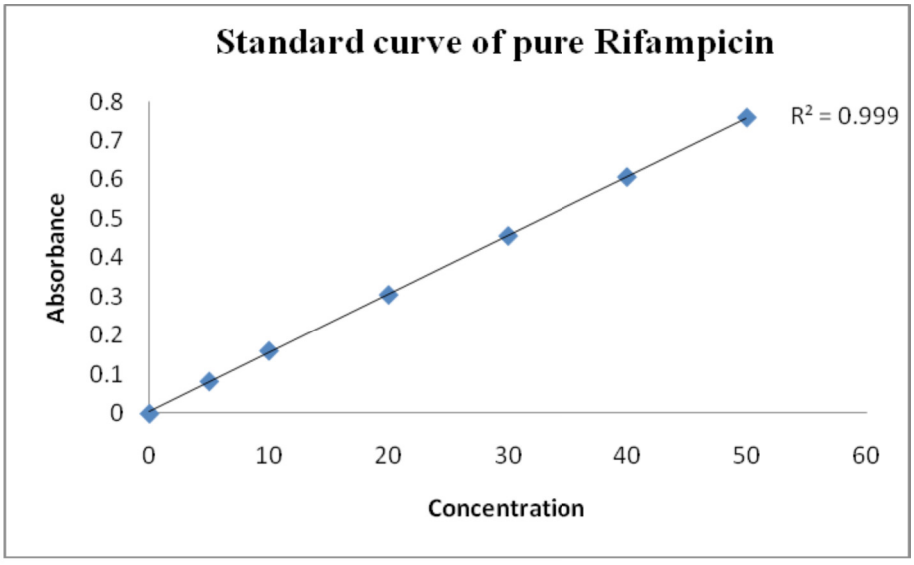


Figure 5

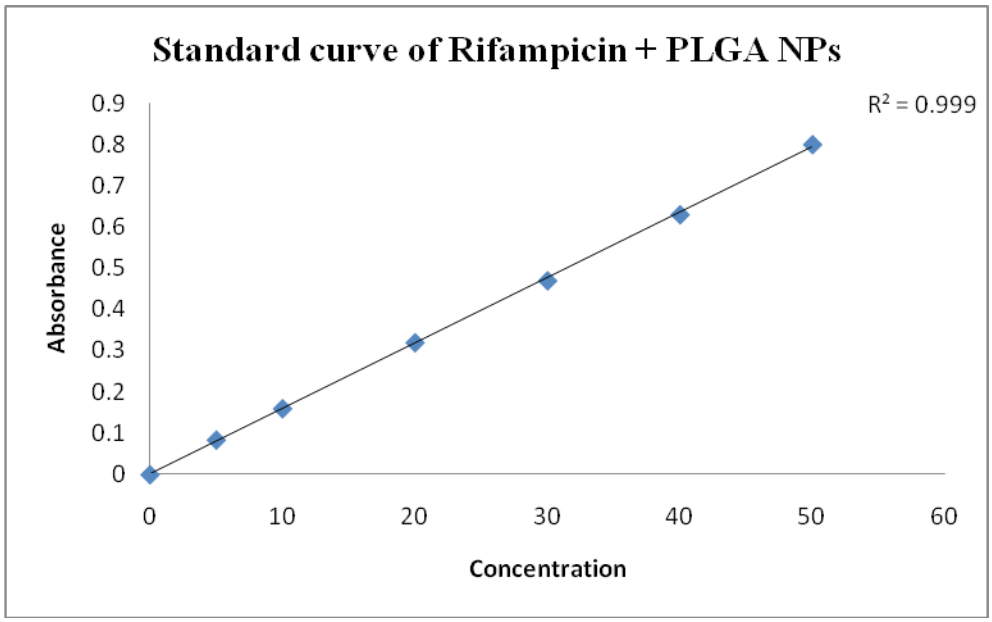


Figure 6

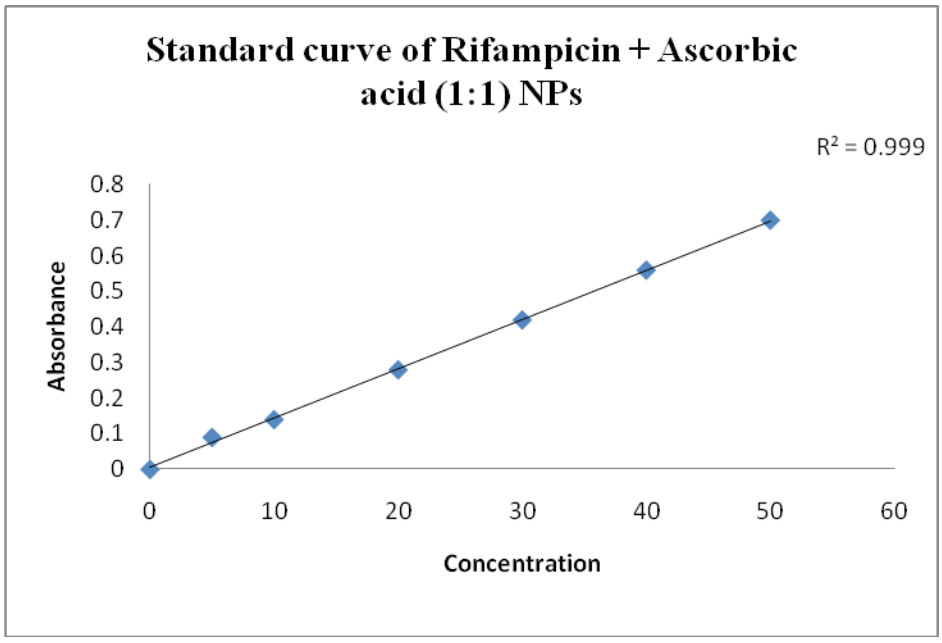


Figure 7

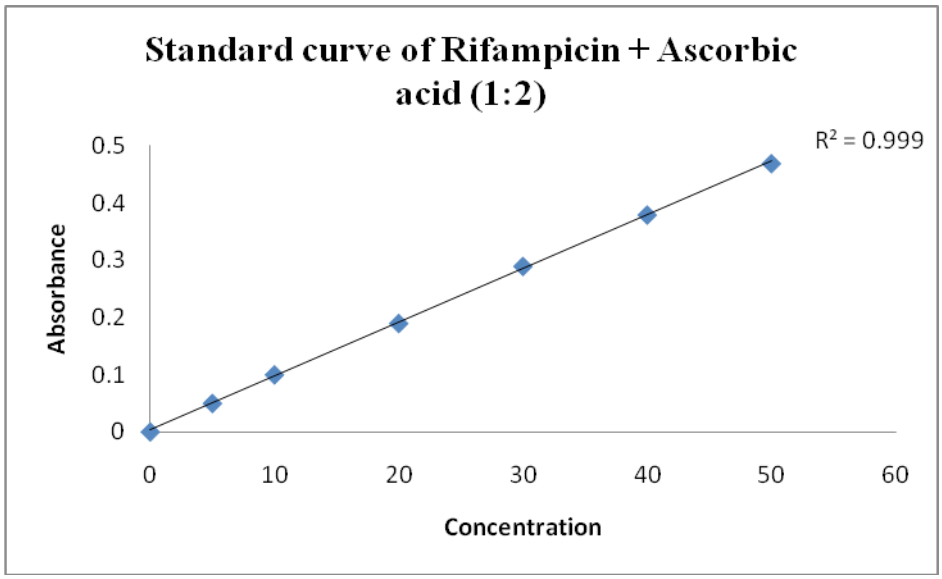
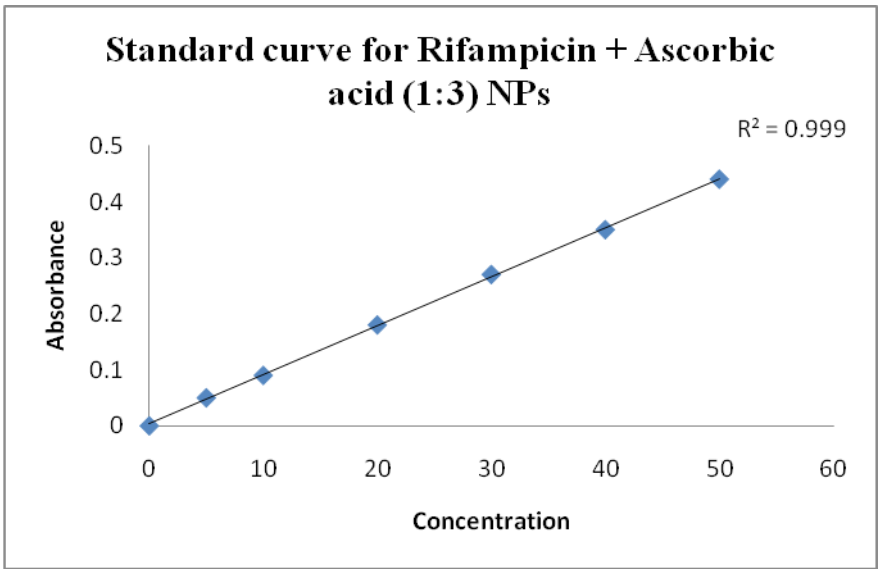


Figure 8

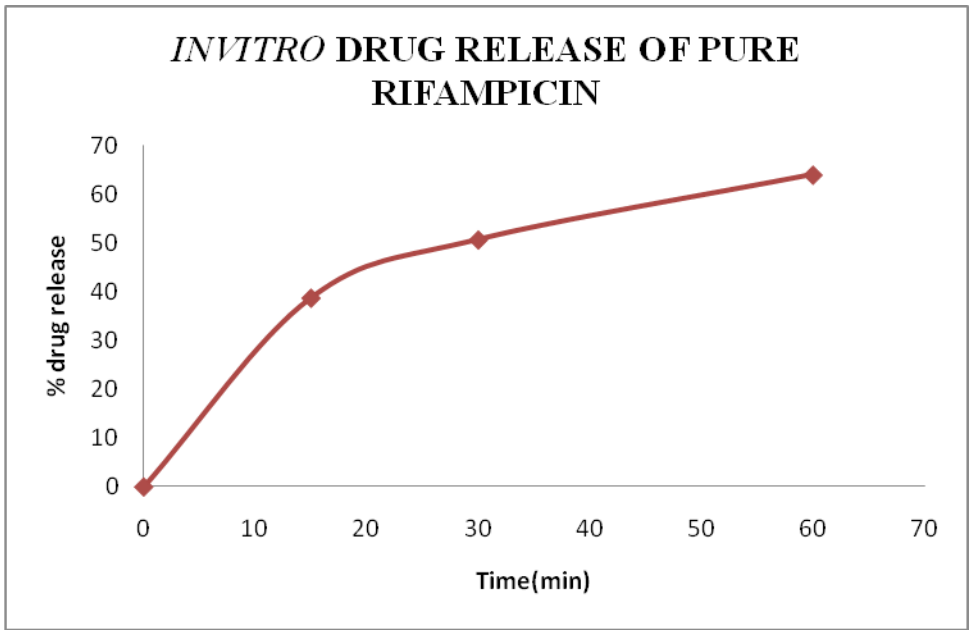


Invitro Stability study of rifampicin PLGA nanoparticles and rifampicin in combination with ascorbic acid in different ratios at pH 1.2 buffer (Table 4 - 8)

Pure Rifampicin (Table 4)

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
0	0	0	0	0	0	0	0	0	0	0
15	0.620	0.621	0.619	43.20	43.21	43.19	38.8	38.81	38.79	38.80±0.010 %
30	0.861	0.860	0.859	56.41	56.40	56.39	50.77	50.76	50.75	50.76±0.010 %
60	0.980	0.980	0.981	71.20	71.20	71.21	64.08	64.08	64.09	64.08±0.005 %
P value										*0.0197

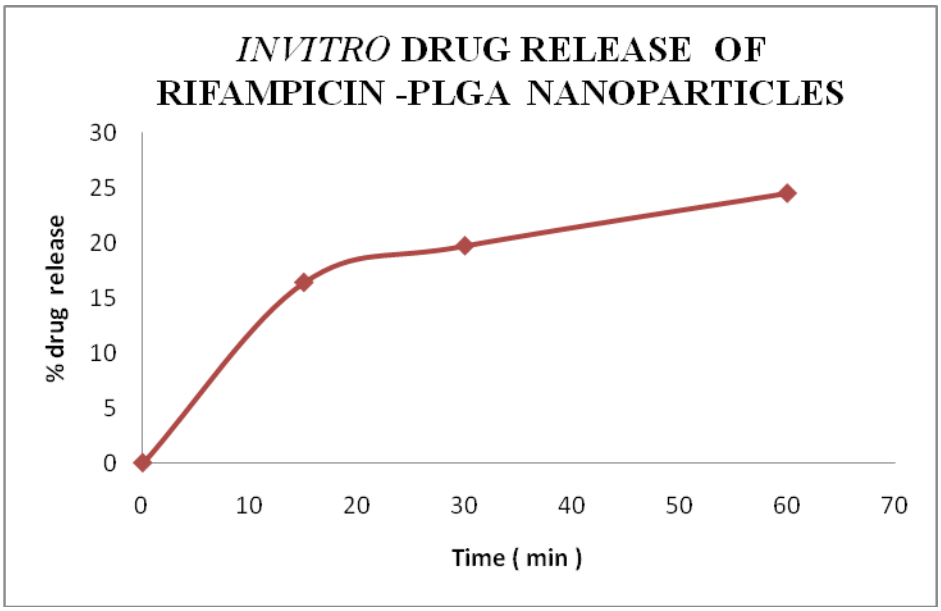
Figure 9



RIFAMPICIN + PLGA NANOPARTICLES (Table 5)

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
0	0	0	0	0	0	0	0	0	0	0
15	0.190	0.202	0.201	18.1	18.2	18.2	16.39	16.42	16.42	16.38± 0.07 %
30	0.335	0.334	0.335	21.90	21.8	21.9	19.71	19.70	19.71	19.71±0.005 %
60	0.420	0.421	0.422	27.20	27.20	27.21	24.48	24.48	24.50	24.48 ±0.011 %
P value										*0.0133

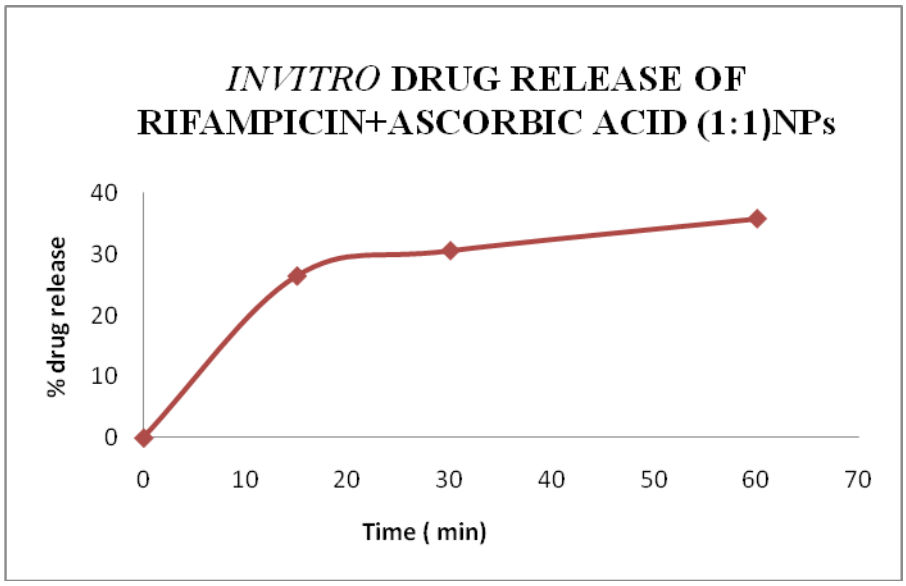
Figure 10



RIFAMPICIN + ASCORBIC ACID (1:1) Table 6

Time (min)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	Trial1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
0	0	0	0	0	0	0	0	0	0	0
15	0.410	0.413	0.412	22.5	29.4	30.0	26.46	26.48	26.48	26.46±0.011%
30	0.472	0.471	0.472	34.20	34.11	34.20	30.61	30.59	30.61	30.60±0.010 %
60	0.540	0.542	0.541	39.80	39.82	39.82	35.82	35.83	35.83	35.82±0.005 %
Pvalue										**0.0076

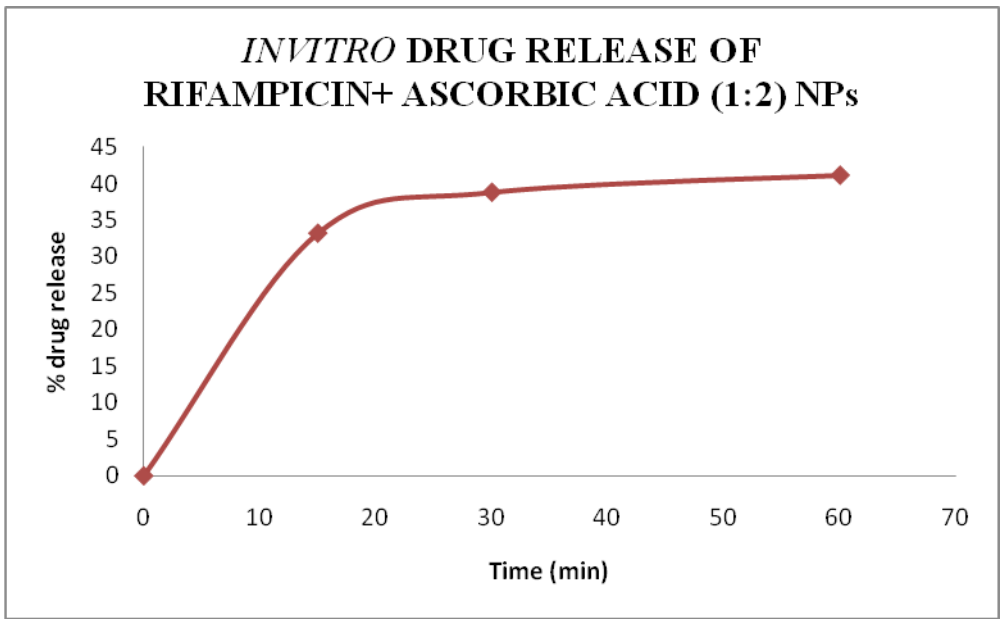
Figure 11



RIFAMPICIN + ASCORBIC ACID (1:2) Table 7

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	Trial1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
0	0	0	0	0	0	0	0	0	0	0
15	0.360	0.359	0.361	37.00	36.90	37.10	33.20	33.19	33.21	33.20±0.010%
30	0.391	0.390	0.389	43.21	43.20	43.14	38.81	38.80	38.79	38.80±0.010 %
60	0.442	0.440	0.440	45.73	45.71	45.71	41.15	41.13	41.13	41.13±0.011%
P value										**0.0039

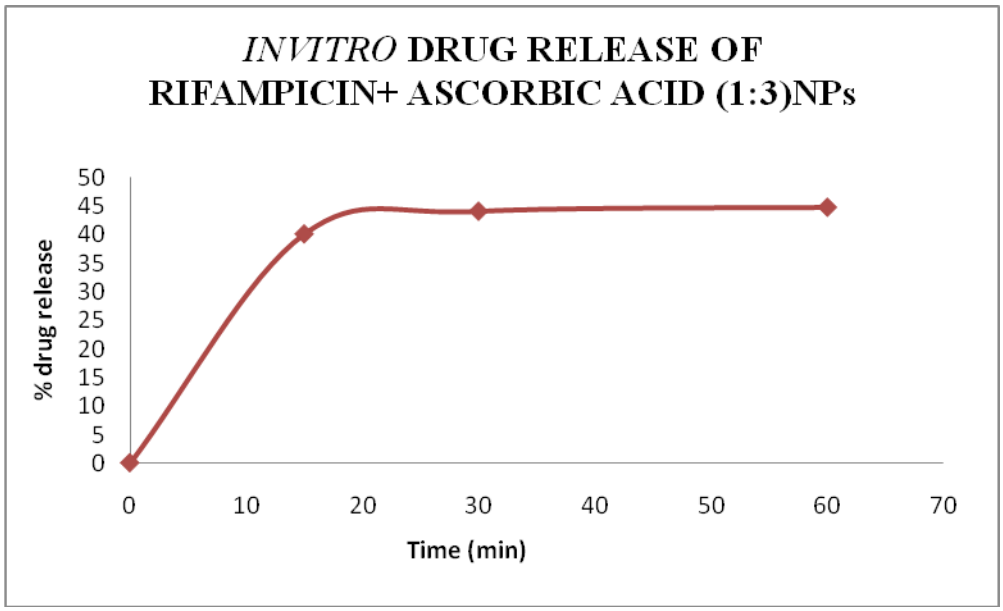
Figure 12



RIFAMPICIN + ASCORBIC ACID (1:3) Table 8

Time (mins)	Absorbance			Concentration			% drug release			Mean % drug release±SD
	Trial1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	
0	0	0	0	0	0	0	0	0	0	0
15	0.351	0.349	0.350	44.71	44.69	44.7	40.21	40.19	40.20	40.20±0.010 %
30	0.442	0.440	0.444	49.20	49.00	49.22	44.20	44.19	44.22	44.20±0.015 %
60	0.446	0.445	0.447	49.90	49.89	49.90	44.89	44.88	44.90	44.89±0.010 %
P value										**0.0011

Figure 13

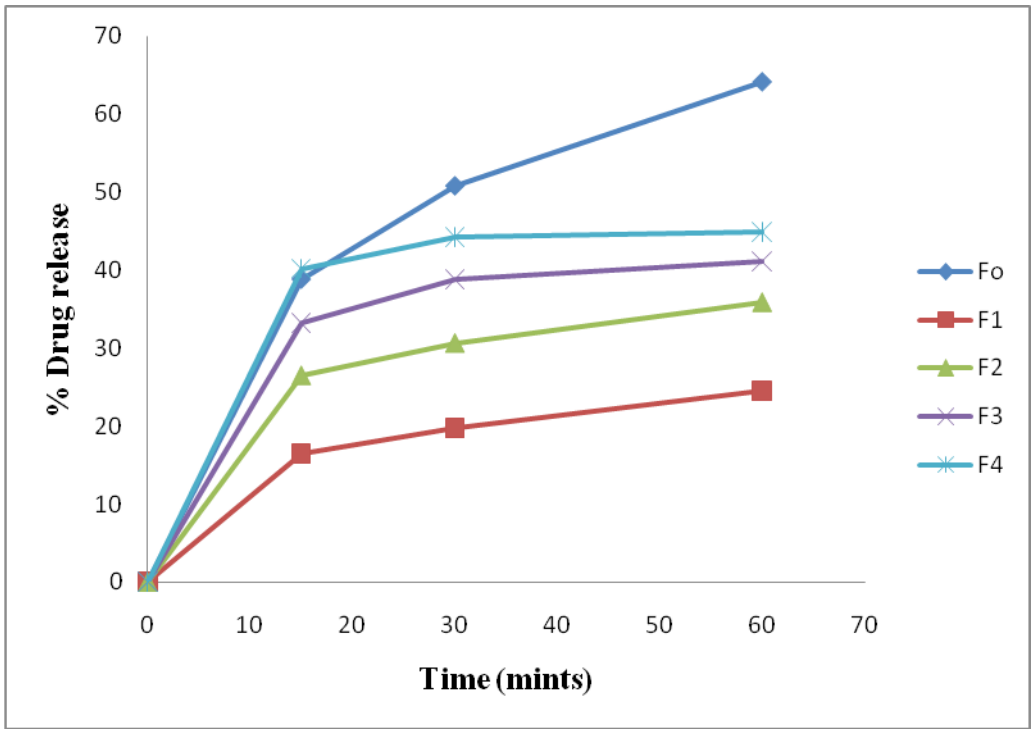


% DRUG RELEASE PROFILE OF THE FORMULATIONS

Table 9

Time (min)	F0	F1	F2	F3	F4
0	0	0	0	0	0
15	38.80±0.010 %	16.38±0.07%	26.46±0.011%	33.20±0.010%	40.20 ±0.010 %
30	50.76±0.010 %	19.71±0.005%	30.60±0.010%	38.80±0.010%	44.20 ±0.015%
60	64.08± 0.005%	24.48±0.011 %	35.82±0.005%	41.13±0.011%	44.89 ±0.010%

% DRUG RELEASE PROFILE OF THE FORMULATIONS Figure 14



Statistical Analysis of all the formulations

One way Analysis of Variance (ANOVA) : **Tukey-Kramer Multiple Comparisons Test**

Table 10

Comparison	P value
F0 vs F1	** P<0.01
F0 vs F2	* P<0.05
F0 vs F3	ns P>0.05
F0 vs F4	ns P>0.05
F1 vs F2	ns P>0.05

F1 vs F3	ns P>0.05
F1 vs F4	* P<0.05
F2 vs F3	ns P>0.05
F2 vs F4	ns P>0.05
F3 vs F4	ns P>0.05
P value	** 0.0020

***** **considered significant**

****** **considered very significant**

Statistical Analysis of nanoparticles

One way Analysis of Variance (ANOVA): **Tukey-Kramer Multiple Comparisons Test**

Table 11

Comparison	P value
F1 vs F2	* P<0.05
F1 vs F3	** P<0.01
F1 vs F4	*** P<0.001
F2 vs F3	ns P>0.05

F2 vs F4 F3 vs F4	* P<0.05 ns P>0.05
Overall P value	* * *0.0005

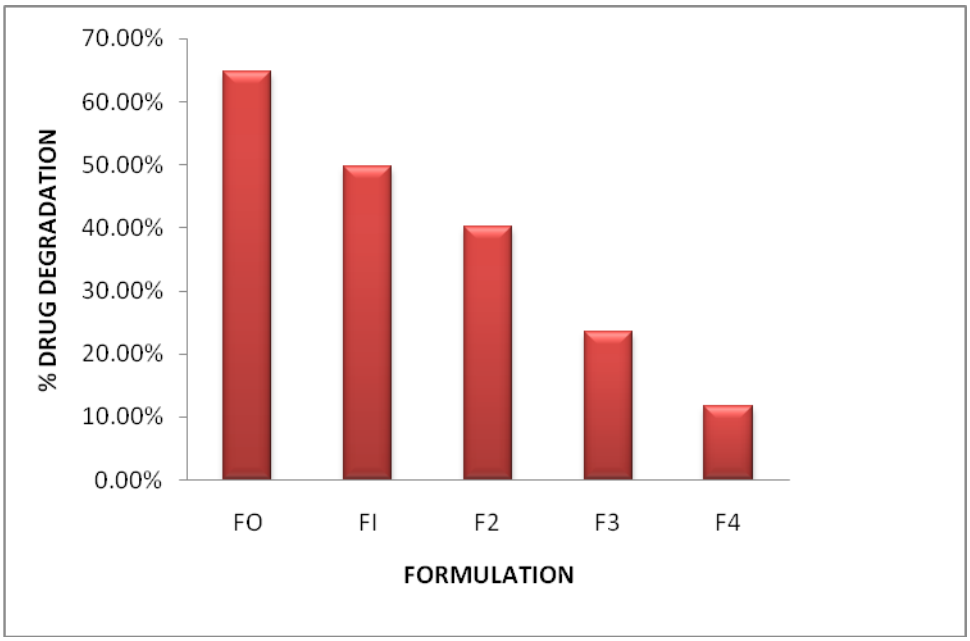
COMPARITIVE % DRUG DEGRADATION PROFILE

Table 12

Formulation Code	% drug degradation
F0	64.81 %
F1	49.77 %
F2	40.17 %
F3	23.54 %
F4	11.61 %
P value	* 0.0156

% DRUG DEGRADATION PROFILE OF F0 TO F4

Figure 15


70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
% DRUG DEGRADATION
FO
FI
F2
F3
F4
FORMULATION

DISCUSSION

11. DISCUSSION

Rifampicin is a first line anti-tubercular drug, administered orally in fixed dose combination with Isoniazid, Pyrazinamide and Ethambutol in order to overcome drug resistance to tuberculosis arising from administration of these drugs separately. However bioavailability of rifampicin is reduced owing to degradation of the drug in the stomach. Rifampicin degrades in acidic condition of the stomach and the degradation of rifampicin is pH dependent.[51] One report says that the problem of poor absorption of rifampicin from combination products is perhaps due to increased decomposition in stomach conditions and the decomposition of rifampicin is enhanced in the presence of INH.[52]

Rifampicin is well absorbed in the pH range of 1-2 even though it undergoes degradation in the acidic medium. Rifampicin hydrolyses to 3 formyl rifampicin SV (3-FRSV)in acidic condition and it undergoes air oxidation in alkaline medium to form in active quinone derivative rifampicin quinine.[53] It shows high antimicrobial activity but is inactive in *in-vivo* (USP DI,1996). Therefore formation of 3-FRSV in the acidic environment of stomach can be an important factor affecting bioavailability of rifampicin and cannot be overlooked.

Development of any method that can prevent or minimize degradation of rifampicin in the stomach either as a single drug or in combination of other anti tubercular drug is therapeutically beneficial and can achieve effective control of tuberculosis with improved bioavailability of rifampicin.

Previous study shows that the degradation of rifampicin due to oxidative side reaction was prevented by the addition of ascorbic acid to the reaction media.[54]Another study reveals the protective effect of adding ascorbic acid on the stability of rifampicin in

plasma and that the degradation can be effectively prevented by adding ascorbic acid thus prolonging stability for 12 hours.[46]

Based on the above factors the present study aimed to prepare and evaluate Rifampicin loaded PLGA nanoparticles and an attempt was made to investigate the influence of ascorbic acid as an antioxidant on stabilizing rifampicin in the gastric environment by *invitro* study in pH 1.2 medium simulating the condition in stomach.

Evaluations of the prepared nanoparticles were then carried out by different methods. Shape and Surface characterization of the nanoparticles were done by Scanning Electron microscopy and the SEM images revealed that the nanoparticles were spherical with smooth surface and the nanoparticles were found to be relatively mono dispersed. Laser particle size analyzer yields the diameter of the bulk population and a polydispersity index gives the distribution range from 0.000 to 0.500. Polydispersity index is a measure of the distribution of particles in a given polymer sample. PdI greater than 0.5 indicates aggregation of particles. Here the polydispersity indexes of the nanoparticles were in the range of 0.308-0.317.

Particles were in the size range of 374 - 380 ± 18 - 23 (SD) nm. Particle size of the nanoparticle can be affected by processing parameters such as drug/polymer ratio, concentration of surfactant and stirring speed. Since in the present study these parameters were maintained constant, their influence on the mean particle size of nanoparticles cannot be ascertained.

Zeta potential is a term related to the stability of samples. For molecules and particles that are small enough, high zeta potential will confer stability i.e. it resist aggregation. When zeta potential is low, attraction exceeds repulsion. Therefore particles with high zeta potential (-ve or + ve) are electrically stabilized. Generally particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. Lower zeta potential facilitates aggregation. Here zeta potential of the prepared nanoparticles was found to be -46.6, which would not allow aggregation.

The invitro dissolution study was conducted for 1 hour. It was carried out using the five formulations. First formulation is pure Rifampicin. Second is rifampicin alone loaded PLGA nanoparticles and in the next three formulations ratio of rifampicin and PLGA were same where as the concentration of ascorbic acid was increased. The dissolution study was carried out in pH 1.2 solutions to simulate the acidic gastric condition. At specific time intervals samples were withdrawn and analyzed by U.V spectrophotometer. Percentage release of drug from the nanoparticles at 15min, 30min and 60min were determined and the percentage degradation of the drug was also calculated.

The results of the invitro drug dissolution study indicates that the % drug release of the formulation F0, F1, F2, F3 and F4 at 60 minutes was found to be 64.08%, 24.48%, 35.82%, 41.13% and 44.89 % respectively and the percentage drug degradation of the formulation F0, F1, F2, F3 and F4 was found to be 64.81%, 49.77 %, 40.17 %, 23.54% and 11.61 % respectively.

From the data obtained it is understood that ascorbic acid minimized the degradation of rifampicin and the degradation was further reduced when the concentration of ascorbic acid was increased. Statistical analysis of the % drug degradation profile was done and it was found that there is a statistically significant change (statistically significant; *P 0.0156) in the percentage degradation as the concentration of ascorbic acid was increased. It can be hypothesized that ascorbic acid being an antioxidant prevents the oxidative side reactions of rifampicin in the gastric pH and minimized the degradation of rifampicin.

SUMMARY AND CONCLUSION

12. SUMMARY & CONCLUSION

The results of the study demonstrate that ascorbic acid can minimize the degradation of rifampicin in gastric pH condition and thus improves the stability and therapeutic efficacy of rifampicin. The study also concluded that there is statistically a significant change in the percentage drug degradation profile when the concentration of ascorbic acid was increased. Further *invivo* studies are recommended to address the therapeutic efficacy of rifampicin –ascorbic acid loaded PLGA nanoparticles

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